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A PROCEDURE FOR GROWING, STAINING, AND MAKING PERMANENT SLIDES OF POLLEN TUBES[1]

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Beatty[2] has recently described a method for growing and making permanent slides of pollen tubes, using egg albumin for an adhesive. The writer in using this method for mitochondrial studies found that fixatives containing bichromates caused the entire smear to come off

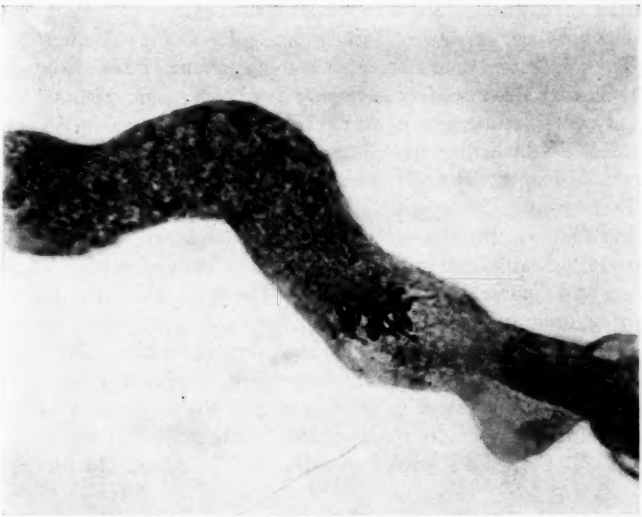

Fig. 1. A portion of a pollen tube of *Amaryllis belladonna*, showing generative nucleus dividing.

the slide. When Nawaschin's fluid is used, as recommended, the technic is still a delicate one, requiring very careful handling of the slides.

[1]Contribution from the Department of Botany, The Pennsylvania State College, No. 107.

[2]Beatty, A. V. 1937. A method for growing and for making permanent slides of pollen tubes. Stain Techn., 12, 13–4.

The following procedure eliminates these difficulties, permitting the use of apparently any killing fluid and fixes the preparation securely enough to the slide to permit normal handling in Coplin jars during staining operations.

One-half gram of agar and an optimum quantity of sugar (2 g. for *Amaryllis belladonna*, 0.5 g. for *Begonia* sp.) are boiled in 25 cc. of tap water or Hoagland's solution. After cooling to about 35° C., or until cool enough to hold the beaker in the hands, 0.5 g. of powdered gelatin is added and the mixture stirred until the gelatin is melted. (If not used immediately, the solution should be kept warm on a warming plate.) This is smeared with the finger in a thin film on the slide (a warmed slide results in a thinner and more even film) and the pollen dusted on. If the pollen is sticky and falls in clumps on the slide it can be spread evenly by smearing it with another drop of the mixture.

The slides are then placed in a moist chamber for germination. They can be removed occasionally for observation, stained supravitally with Janus green or neutral red, or killed when desired in any suitable fluid. Killing for 8 hours or overnight and washing for 2–4 hours in cold, running water seems adequate.

For staining, the crystal violet procedure originated by L. F. Randolph for root tip chromosomes is recommended. It may be followed by a counterstain of 1% gold orange in clove oil for differentiation and clearing of pollen tubes. The accompanying figure is a photomicrograph of a portion of a pollen tube of *Amaryllis belladonna* stained by this method, showing the generative nucleus dividing.

The following is the staining procedure, used and published with the kind permission of L. F. Randolph, with certain very minor changes made necessary for its adaptation to pollen tubes:

1. 1% potassium permanganate, 2–3 minutes. Rinse in tap water.

2. 5% oxalic acid, until the sections are bleached, usually 1–3 minutes. Wash in tap water 15 minutes. Bleaching in potassium permanganate and oxalic acid is not always necessary but it usually adds contrast.

3. Mordant in 1% chromic acid, 20 minutes. Rinse in tap water and then in 2 or 3 changes of distilled water.

4. 1% aqueous solution of crystal violet, 4 hours. The staining period may be altered according to the time required for de-staining.

5. Treat with iodine-potassium iodide (iodine, 1 g.; potassium iodide, 1 g.; in 100 cc. of 80% alcohol) until the color of the sections changes from blue to brown, usually 1–2 minutes.

6. Rinse in 95% alcohol and apply counterstain of 1% gold orange in clove oil until differentiated, usually 2–4 minutes.

7. Rinse in 2 changes of absolute alcohol, clear in xylene and mount in balsam.

Randolph does not use a counterstain in his original method for root tip chromosomes. From 6 above his procedure is as follows:

Rinse in 95% alcohol and pass thru 3 changes of absolute alcohol to clove oil. Differentiate in the alcohols and clove oil, ordinarily1–3 minutes. Watch the process in the final stages under the microscope. The metaphase chromosome groups under a 16 mm. objective should stand out sharply against a practically colorless background of cytoplasm.

Pass thru several changes of xylene to remove all of the clove oil. Mount in thin xylene-balsam. After the cover glass is in place invert the slide on paper toweling and apply mild pressure to force the excess balsam from under the cover glass. Add a few drops of xylene to the edges of the slide, cover with another paper towel and a piece of heavy glass, or other suitable weight. As soon as the slides are dry they may be examined. This method of mounting removes all excess balsam and brings the cover in close contact with the material so that high-power objectives may be used with greater safety.

The technic described above has also been used successfully for germinating fungi spores and for cytological observations on mycelium.

A CONTROLLABLE DIFFERENTIAL STAIN FOR THE HYPOPHYSIS BY ADAPTATION OF THE MALLORY TRIPLE STAIN

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ABSTRACT.—**A selective and controllable staining method for the hypophysis has been developed with rat material, using Mallory's triple stain as a basis.**

Fix in Zenker neutral formol for 6 hours. Longer fixation is undesirable. Transfer to 30% alcohol plus a few drops of a saturated solution of I_2 in aqueous KI over night. Gradually complete dehydration and clear in cedar oil. Infiltrate with a paraffin mixture (paraffin, rubber-paraffin, bayberry wax and beeswax). Section 3–5μ. Hydrate to distilled water, placing a few drops of a KI–I_2 solution in the 50% alcohol. Stain in 1% acid fuchsin for 30 minutes. Rinse, and differentiate in a weak NH_4OH solution (one drop 28% NH_4OH to 200 cc. HOH). When differentiation is complete, transfer to a 0.5% phosphomolybdic acid solution for 3 minutes, after first stopping the differentiation with a 0.1% HCl solution and then rinsing with distilled water. Stain for one hour in a solution of: 1% anilin blue, water soluble, 2% orange G, and 1% phosphomolybdic acid. Rinse in distilled water plus a few cubic centimeters of the stain. Differentiate in 95% alcohol, transfer to absolute alcohol and clear in a mixture of 30% oil of cedar, 40% oil of thyme, 15% absolute alcohol and 15% xylene. Finally, transfer to xylene and mount.

Most of the methods commonly employed for differential staining of the hypophysis are variable in the results produced because the fixation of the dyes to the chromophilic elements is difficult to control. This is particularly true in the use of Mallory's triple stain and its various modifications; first, because the partial decolorization of the acid fuchsin is not carried out under controlled conditions; second, the rapid dehydration and differentiation of the second stain permit no refined differentiation of the basophilic elements. This paper describes in detail a modified Mallory method which eliminates these difficulties and permits one to control the degree of staining of both the eosinophilic and basophilic elements under the microscope.

The technic described below was originally developed using a Holl-

born sample of water blue (Wasserblau, H-274)[1] as the stain for basophiles. This was employed under the assumption (after an apparent failure in using the Mallory technic without the differential features described below) that it was a higher homolog of the triphenylrosanalin series than anilin blue, water soluble. It was, however, pointed out by H. J. Conn that theoretically the two names are synonymous. Samples of certified anilin blue W. S. were furnished by the Stain Commission, and proved to give the same results as the Hollborn dye. It is not, accordingly, a question of the dye, but one of controlled differentiation of the staining.

The gland is removed as quickly as possible and fixed not longer than 6 hours in Zenker formol, in which the 40% formalin stock solution was neutralized with $MgCO_3$ and added to the $HgCl_2$-$K_2Cr_2O_7$ solution in a proportion of 5:95 just before using. Fixation for a longer period is not desirable since the differential staining properties of the tissue are affected. The tissue is then rinsed with distilled water and transfered to a solution of 30% alcohol plus iodine over night. The iodine is made up as a stock solution by adding enough crystals of I_2 to a 2% KI solution to saturate it. A few drops of this stock solution are added to the 30% alcohol until a clear light, brownish orange solution is obtained. The tissue is then dehydrated over a period of 4–5 hours and cleared over night in cedar oil. Prolonged immersion in the cedar oil does not seem to affect the tissue and storage is possible at this stage. It is more advisable, however, to embed and then store the blocks until ready for use. The glands are placed in xylene for 15 minutes and then infiltrated with a paraffin mixture of the following composition for one hour:

56–58° paraffin	100 g.
Rubber paraffin	4–5 g.
Bayberry wax	5–10 g.
Beeswax	1 g.

The glands are sectioned at 3–5 μ and run thru the usual hydration series. A few drops of the KI-I_2 solution are added to the 50% alcohol to remove any Hg which might have remained in the tissue. From the distilled water the sections are transferred to a 1% aqueous acid fuchsin solution and allowed to remain there for 30 minutes. The sections are then rinsed in tap water. Differentiation of the eosinophiles is carried out in a dilute solution of NH_4OH (1 drop of 28% NH_4OH to 200 cc. distilled water) by dipping the slide in and out of the solution until the sections become a pale pink. The degree

[1]Referred to as "Grübler water blue H-274" in letter from Pfaltz and Bauer, Nov. 18, 1937.

of differentiation is controlled by dipping the slide in a 0.1% aqueous HCl solution, which stops the decolorizing process and then examining the slide under the low power of the microscope. The slides are placed upon an old photographic plate, from which the emulsion has been removed; this keeps the stage of the microscope from becoming wet. If the differentiation is not complete, one returns to the NH_4OH solution until by such repetition of the process the desired degree of differentiation is obtained. This procedure is preferable to the technic of immersing the slide in the differentiating fluid contained in a Petri dish. By using the former procedure it is possible to carry out the process until the eosinophilic granules are distinct. (In interpreting this result, however, one must consider the mitochondria which are also stained. Severinghaus[2] has developed an excellent method for especially differentiating the mitochondria from the eosinophilic granules after fixation in a modified Champy's fluid.) After the HCl bath the sections are transferred to a few changes of distilled water and then placed in a 0.5% phosphomolybdic acid solution for 3 minutes. The slides are then transferred to a solution of the following composition for one hour or longer.

Anilin blue, water soluble	1 g.
Orange G	2 g.
Phosphomolybdic acid (yellow crystals)	1 g.
Distilled water	100 cc.

The sections are then washed in distilled water to which has been added a few cubic centimeters of the staining solution, thus preventing the stain from differentiating too rapidly. The sections are then dipped in 95% alcohol several times, then into absolute alcohol, and finally examined under the microscope. After a little experience, the end-point of the differentiation is easily recognized and a refined differentiation can be made by using the microscope. *It is essential that a standard source of light be used with the microscope since the blue appears diffuse with daylight.* A substage lamp serves this purpose well. After a satisfactory end-point is obtained, the sections are transferred to a clearing mixture of the following composition:

Oil of cedar	30 cc.
Oil of thyme (red)	40 cc.
Xylene	15 cc.
Absolute alcohol	15 cc.

This mixture serves two purposes: first, to complete the dehydration so that the sections will be perfectly clear under the oil immersion

[2]Severinghaus, A. E. 1932. A cytologic technique for the study of the anterior lobe of the hypophysis. Anat. Rec., **53**, 1.

lens; second, in a finer technical procedure it eliminates radical diffusion currents. The sections are allowed to remain here for about five minutes. They are then transferred thru two changes of xylene and are mounted under cover slips, using neutral gum dammar.

The writer is of the opinion that the method described above is of particular value to workers engaged in histophysiological problems, either those involving direct observations or those in which cellular change is accompanied by experimentally-produced conditions in other endocrines. The method is easily applicable and offers especial value in comparative studies by virtue of the controlled differentiation feature. This method was used on the hypophysis in conjunction with thymus studies and X-ray irradiation in the albino rat.[3]

The author is indebted to Dr. I. Zeckwer, of the Pathological Department at the University of Pennsylvania, for the kindly examination and criticism of his preparations, and especially to Dr. David R. Meranze for his interest and the facilities afforded him in the Histological Laboratories at the Mount Sinai Hospital. He is likewise indebted to Dr. H. J. Conn, chairman of the Commission on Standardization of Biological Stains, for his whole-hearted cooperation.

[3]Gershon-Cohen, J., Meranze, D. R., Shay, H., Meranze, T., and Fels, S. 1938. Studies in the physiology of the thymus. Science, **87**, 20. The Thymus: The effect of atrophy of the thymus following Roentgen irradiation. Amer. J. Roentgenology, **39**, 263–70.

AN INEXPENSIVE MICROSCOPE LAMP FOR CRITICAL ILLUMINATION

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Abstract.—**A microscope lamp with light of adjustable intensity, which will afford critical illumination of a quality satisfactory for the most exacting cytological work, can be constructed for about $7.50. The essential parts needed are: auto headlight bulb, radio power transformer, radio "potentiometer", and simple plano-convex lens. A ground glass is not used with 4 and 2 mm. objectives, a solid source of light being obtained by placing the bulb so that one filament coil is directly behind the other. This light source is brought to a focus by the condensing lens at a field diaphragm and this image is projected by the microscope condenser into the plane of focus of the microscope.**

There are two main types of microscope illumination in general use in cytological research at the present time. In one type the light from the bulb filament is diffused either by a frosted bulb or by a ground glass interposed between it and the microscope condenser; in the other the image of the bulb filament is projected by the microscope condenser into the plane of the object and, therefore, into the plane of focus of the microscope. The latter has been gaining much favor among cytologists since the appearance on the market of the ribbon filament bulb. The costliness of this kind of bulb, the fact that it is relatively short-lived, and the expense of accessory equipment, such as a transformer of high amperage capacity and a high voltage rheostat, however, place this outfit beyond the financial means of many microscopists.

The lamp described below is of this type, but requires less expensive parts for its construction, viz., coiled filament bulb, radio power transformer, and radio rheostat. The total cost, including condensing lens, is about $7.50. The writer has had such a lamp in constant use for over a year. It has been subjected to critical examination by several cytologists at the Department of Genetics of the Carnegie Institute of Washington, who have pronounced it satisfactory for the most exacting cytological work.

The arrangement of the parts is shown in Fig. 1. The bulb (B) is a 50 c. p. 6–8 v. single filament auto headlight bulb held upright in a

socket that is fastened immovably to the bottom of the lamp box (LB). It is so placed that one filament coil is directly behind the other in relation to the microscope in order to eliminate any dark areas that might otherwise appear between successive turns of the spiral coils. The socket terminals are connected to the 6.3 v. 2 amp.[1] leads (b) of the transformer (T) (Jefferson power transformer No. 463–381). One of these wires is intercepted by a 2-ohm 50-watt radio "potentiometer" (P) connected as a resistance. This bulb gives a light so intense that, even with a dense filter, it is rarely necessary to turn the "potentiometer" more than four-fifths on. The 120 v. wires (a) of the transformer are connected thru a switch (S) on the transformer box (TB) to the 120 volt alternating city current by a wall plug (W). Since both bulb and "potentiometer" generate heat it is important that the boxes in which these are placed contain many openings for air circulation. As a material for their construction ¼ inch veneer recommends itself, because it is easy to work and is not warped by the heat.

The condensing lens (L) is a simple plano-convex lens of 19 mm. equivalent focal length (Bausch & Lomb No. 61–44–30–012). The plane surface is directed toward the bulb. It rests in a grooved semicircular support that is adjustable for height and for distance from bulb, since its base fits between two strips of wood in the bottom of the lamp box. It is so placed that the rear filament is brought to a focus at the field diaphragm (F), i. e., an old iris diaphragm mounted on an adjustable support (AF). Measured from its plane surface, the above lens should be about 19–20 mm. from the center of the bulb and about 170 mm. from the field diaphragm. The distance from diaphragm to microscope may be varied within wide limits; 30–50 cm. has proved to be most satisfactory.

The method of setting up a similar system has been described by Bridges.[2] Bulb filament, lens, and field diaphragm must be in a direct line with the microscope mirror (M). With the field diaphragm wide open, raise or lower the lens until the approximate middle of the bulb filament image coincides with the center of the mirror. This may be readily checked by observing the reflection of this image on a dark card or ground glass held against the mirror. Look thru the microscope with the 16 or 8 mm. objective focused on

[1]Altho these leads are marked 2 amps., data on the winding diagram of this transformer indicate that these wires are large enough to carry a current of 9 amps., which is more than enough for the 50 c. p. bulb used.

[2]Bridges, C. B. 1936. The examination of salivary chromosomes. Drosophila Information Service, Carnegie Inst. of Washington, Cold Spring Harbor, N. Y., No. 6, 37–40.

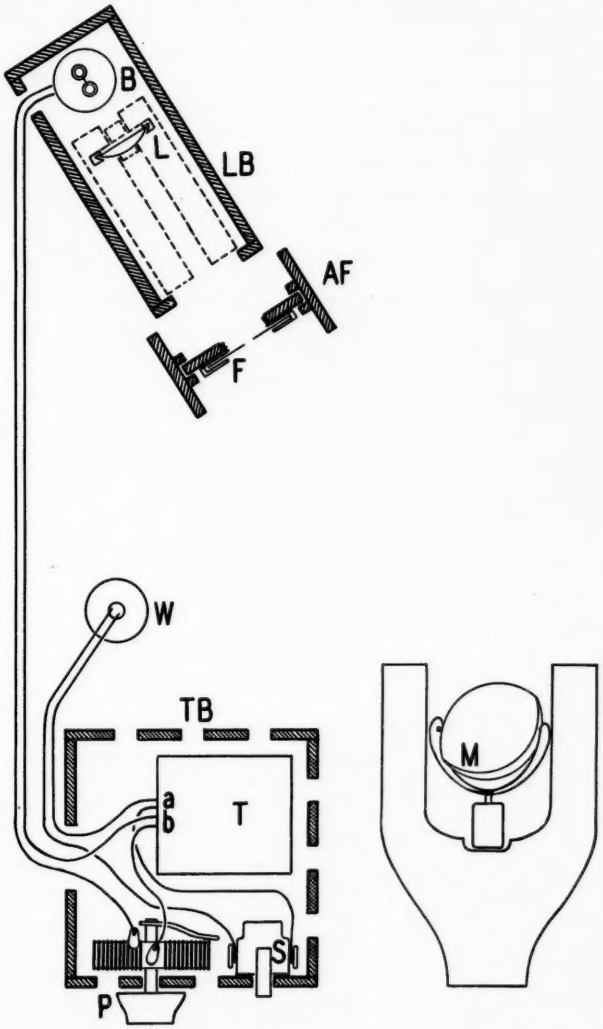

Fig. 1. Diagram of lamp. AF, adjustable support for field diaphragm; B, bulb; F, field diaphragm; L, condensing lens; LB, lamp box; M, microscope mirror; P, "potentiometer", connected as resistance; S, switch; T, transformer; TB, transformer box; W, wall plug; a, 120 v. wires; b, 6.3 v. 2 amp. wires.

an object and adjust the mirror so that the bulb filament image lies in the middle of the field. Then close the field diaphragm to a pin hole and raise or lower the condenser until the edges of the diaphragm are in sharp focus in the field. If the centers of the field diaphragm image and the microscope field do not coincide, move the diaphragm from side to side or up and down until they do. Check this arrangement with the 2 mm. lens and, if necessary, readjust the condenser and field diaphragm. If the alignment of all the parts is now correct, the field diaphragm opening should lie in the middle of the bulb filament image as seen on the back of the diaphragm.

With 2 and 4 mm. objectives the field diaphragm opening should be about $\frac{1}{4}$–$\frac{3}{8}$ inch in diameter, so that the image of the edges is not quite visible in the field of the microscope. With 8 and 16 mm. lenses, however, it is necessary to lower the condenser or insert underneath it a ground glass—preferably ground on both sides—in order to obtain a completely and uniformly illuminated field.

Inexpensive but highly satisfactory light filters can be made by mounting in balsam or dammar between two glass slips one or more layers—depending on the density desired—of green, blue, yellow, or red cellophane.

DEMONSTRATING WEAKLY ACID-FAST TUBERCLE BACTERIA IN SPUTUM

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ABSTRACT.—**Acid-fastness of the tubercle bacteria has long been used as the common method of diagnosis in sputum. It has been suggested sometimes that tuberculosis could occur without demonstrable bacteria, as well as with acid-fast bacteria, non-acid-fast bacteria or granules. It is shown in this paper that some of the sputa which are negative to the standard staining technic will show rods, rods with round polar bodies, or similar bodies without the rod portion. It is also pointed out that the decolorization of the smears by acid alcohol be shortened to approximately 3 to 5 seconds and picric acid be used as a counterstain. These forms are apparently the varying stages of the loss of acid-fastness. It is essential that a counterstain be used which will not interfere, and yellow is indicated because it does not absorb the red rays. Sputa which are negative to the standard acid-fast staining technic but which come from persons with a variable intermittent fever should be stained by this modified technic before they are pronounced germ-free.**

The presence of acid-fast organisms of the *Mycobacterium* group in sputa, etc., has been taken as a presumptive test for tuberculosis. The chance of failure or a mistaken diagnosis has not been overlooked, consequently, refinements in technic have been proposed from time to time to change presumptive negatives to positives and to separate the smegma, lepra and other acid-fast organisms. When no organisms are found after diligent search microscopically and the specimen is pathogenic for guinea pigs, the conclusion may be either that decolorization of the acid-fast organisms took place or that non-acid-fast tubercle organisms were present.

Malassez and Vignal (1883) were probably the first to suggest a tuberculosis without organisms. Similar suggestions were recorded later by Tecon (1911), Remlinger (1928) and Durand, Kourilsky and Benda (1928). Much (1907) using his modification of the Gram stain did not agree to the theory of tuberculosis without organisms but suggested that tuberculosis might be caused by any one of three forms, (a) acid-fast bacteria, (b) non-acid-fast bacteria or (c) granules. Van Gieson (1911) believed that the granules of Much were not related in any way to tuberculosis. Tint and Breskman (1910)

concluded from their investigations that no decolorants were available which would demonstrate tubercle bacteria in all cases.

Since it is not possible to have animal inoculation on all cases of suspected tuberculosis, prime importance must be given to the method used in decolorizing and counterstaining to obtain the greatest number of positive findings.

A case which came before the author for microscopic diagnosis presented the points under discussion. The patient reported that he had had tuberculosis and had been previously pronounced an arrested case. At the time of examination he showed a temperature fluctuation from normal on some days to slightly above 99° F. on other days. The physical signs and an x-ray picture indicated an advanced case of tuberculosis, however, no tubercle bacteria could be found by the standard staining technic. The microscopic findings, considered without further checking, indicated this to be a case of tuberculosis without demonstrable organisms. Guinea pig inoculation which was done after the technic of this report was devised showed the presence of tubercle bacteria.

The staining technic in the study of this case was changed by decreasing the decolorizing time, using picric acid as a counterstain. The method follows:

Stain with steaming Czaplewski's carbol fuchsin 6–10 minutes, wash well, decolorize in 3% HCl in 70% ethyl alcohol approximately 3–5 seconds, counterstain with either an aqueous or alcoholic solution of picric acid. The decolorization is the most important part and it must be remembered that an alcoholic solution of picric acid can replace the acid alcohol as a decolorant. For that reason if the alcoholic solution be used, more care must be taken to prevent excessive decolorization. The decolorization is carried to the point where the majority of the other bacteria in the smear are decolorized, however, a small percentage of them will still show a very faint pink color. This stage of decolorization is obtained by dipping the slide into a staining jar of acid alcohol, removing it immediately, washing under the tap and repeating the process two or three times. The number of times it was found necessary to dip the slide into the acid alcohol varied somewhat depending upon the age of the acid alcohol and the thickness of the smear.

The microscopic appearance of the tubercle organisms was atypical. The rods were long and slender with distinctly redder round bodies immediately at each end. These bodies did not change the size or shape of the cell in any way and gave the appearance of being a spore at each end of the cell. In slides which had been decolorized very slightly more, these bodies were still visible but the remainder of the

tubercle cell, being less acid resistant, had been completely decolorized. There was no danger of confusing the bright red color of the round bodies or the lighter red of the connecting rod with the very light pink of the other bacteria.

In a series of tests made on the above case on different days, the same picture was obtained. In cases of normal tubercle bacteria, decolorization could be carried out by placing the slide in the staining jar and leaving it for some minutes without decolorizing the organisms.

Sputa were obtained at a later date from eleven cases at the District of Columbia Tuberculosis Sanatorium thru the courtesy of the director, Dr. J. W. Peabody. None of these patients showed tubercle bacteria by the standard staining technic and no history of the case was given at the time the sputa were obtained. Five of the eleven sputa were positive by the method outlined above. Two of these five sputa showed rods and round bodies which retained the stain slightly less than the organisms of the case described above. These two cases might be classed in an empirical manner as +++ reactions using the original case as a ++++ reaction. Both of these patients showed a variable intermittent fever similar to the original case. Two cases would be designated as + and one case as + —. The granules only, or the granules with very faint outlines of the rods, were noted in the least positive reactions. None of the last three cases showed a temperature above normal. In the six negative cases the patients had shown no temperature for some time. It was not possible to obtain guinea pig inoculation with these 11 cases.

It appears entirely probable that these polar bodies are the granules of Much since they are more acid-fast than the remainder of the cell and are approximately the same size. Rochaix and Colin (1911) have shown that Much's granules hold the Gram and Much stains distinctly longer than the rods which agrees with the acid alcohol resistance of the bodies described here.

The color of the background was one of the deciding factors which enhanced visibility of the rods and the round polar bodies. Hauser (1898) was probably the first to substitute picric acid for sulfuric or nitric acid as a decolorant and obtain a good counterstain at the same time. Tarchetti (1904 a and b) and others[1] using picric acid and Doglio (1932) and Margolena (1933) using brilliant yellow as the counterstain have shown one or more of the following advantages: a higher percentage of positives, better visibility with less eye strain and a greater number of organisms. Lancelin, Seguy and Dubreuil

[1]See Bender (1921), Böhm (1912), Jötten and Haarman (1920) Pottenger (1931), Schulte-Tigges (1920), Simmons and Steves (1926), Spengler (1907).

(1926) recommended picric acid for thick smears. Corper (1926) and Matheson and Ayyar (1930) recorded better results with the Ziehl-Neelsen method.

One reason why better results have been obtained by various investigators who have used a yellow counterstain is founded upon a law of physics. Red and orange light rays are absorbed in passing thru blue but are not absorbed by yellow. The background when stained blue would tend to absorb the red color of the organisms and also the outer layer of the cell may be stained slightly blue, especially if the organisms have a tendency toward non-acid-fastness. Yellow, therefore, makes an ideal counterstain because it does not absorb red light.

Since the organisms from the whole sputum may be pathogenic for guinea pigs, as shown by the first case, it would indicate the presence of an undetermined pathogenic form. In order to determine whether the less acid-fast forms, the non-acid-fast forms or the granules will produce characteristic lesions, as Much (1907) and Terebinsky (1908) thought, an infallible separation of these various forms must be made. Such separation would be quite difficult if not impossible in sputum. There can be no doubt that many cases of this type have been pronounced germ-free with the subsequent spreading of the disease.

The results in this report indicate that when diagnosis is dependent upon microscopic technic a yellow counterstain should be used and that sputa which are negative to the standard acid-fast staining technic but which come from persons with a variable intermittent fever should be stained by this modified technic before they are pronounced germ-free.

REFERENCES

Bender, W. 1921. Zur Technik des Nachweises der Tuberkelbacillen im Sputum. Centbl. Bakt., I Abt. Orig., **86**, 461.

Böhm, J. 1912. Über die Verschiedenen Färbemethoden der Tuberkelbacillen deren kritische Rezension. Centbl. Bakt., I Abt. Orig., **62**, 497.

Corper, H. J. 1926. Methods of staining tubercle bacilli. J. Lab. & Clin. Med., **11**, 503.

Doglio, P. 1932. Su un nuova metodo di colorazione del bacillo di Koch nell 'espettorato. Giorn. Batt. Imm., **8**, 243.

Durand, H., Kourilsky, R. and Benda, R. 1928. Possibilité d'une étape abacillaire mais virulent en période latente de tuberculose. Compt. Rend. Soc. Biol., **99**, 32.

Hauser, G. 1898. Note sur la coloration du bacille de la tuberculose. Compt. Rend. Soc. Biol., Series 10, **5**, 1003.

Jötten, K. W. and Haarman, P. 1920. Neure Färbungsverfahren für Tuberkelbazillen. Münch. Med. Woch., **67**, 692.

Lancelin, Seguy and Dubreuil. 1926. Nouvelle technique de recherche du Bacille de Koch. Compt. Rend. Soc. Biol., **94**, 519.

MALASSEZ, L. and VIGNAL, W. 1883. Tuberculose zoogloéique. Arch. physiol., Series 3, **2**, 369.

MARGOLENA, L. A. 1933. Notes on the staining of tubercle bacilli in sputum. Stain Techn., **8**, 73.

MATHESON, D. C. and AYYAR, K. K. 1930. Studies on the staining of acid-fast bacteria. Indian Vet. J., **6**, 187.

MUCH, H. 1907. Über die granuläre, nach Ziehl nicht färbbare Form des Tuberkulosevirus. Beitr. Klin. Tuberk., **8**, 85.

POTTENGER, J. E. 1931. The demonstration of rare tubercle bacilli in sputum. Amer. Rev. Tuberculosis, **24**, 583.

REMLINGER, P. 1928. Peut-il exister une phase abacillaire virulent á la période ultime de la tuberculose pulmonaire? Compt. Rend. Soc. Biol., **99**, 278.

ROCHAIX, A. and COLIN, G. 1911. Coloration du bacille tuberculeux et granulations de Much. Comp. Rend. Acad. Sci., **153**, 1530.

SCHULTE-TIGGES, H. 1920. Zur Tuberkelbazillenfärbung. Deut. Med. Woch., **46**, 1225.

SIMMONS, J. S. and STEVES, E. J. 1926. A comparison of the Schulte-Tigges and the Ziehl-Neelsen methods for staining acid-fast bacteria. Amer. Rev. Tuberculosis, **14**, 102.

SPENGLER, C. 1907. Neue Färbemethoden für Perlsucht- und Tuberkelbacillen und deren Differentialdiagnose. Deut. Med. Woch., **33**, 337.

TARCHETTI, C. 1904a. Per la ricerca dei bacilli tubercolari. Cron. clin. med. di Genoa, **10**, 164.

———— 1904b. Per la ricerca dei bacilli tubercolari. Gazz. Osp., **25**, 707.

TECON. 1911. Les tuberculoses pulmonaires "sans bacille". Rev. méd. Suisse Rom., **31**, 797.

TEREBINSKY, W. J. 1908. Sur la signification des formes non acido-resistants du bacille tuberculeux. Ann. dermat. syph., Series 4, **9**, 503.

TINT, L. J. and BRESKMAN, L. 1910. A study of acid-fast and acid-semifast tubercle bacilli (human). J. Amer. Med. Assoc., **54**, 1591.

VAN GIESON, I. 1911. Acid-fast particles associated with the tubercle bacillus. Collect. Stud. Research Lab., Dept. Health, N. Y., **6**, 265.

THE DEMONSTRATION OF RETICULOCYTES WITH WRIGHT'S STAIN[1]

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In the course of studying supravital preparations made from blood infected with *Plasmodium vivax* it was noted that when Wright's stain was used the reticulocytes were readily demonstrable. Since no record of such a use for this dye has been found it was thought worth while to record these observations.

A 0.3% solution of the stain in absolute, acetone-free methyl alcohol was used and in making moist preparations a thin, even film of the dye was spread on a glass slide. A small drop of blood was placed on a cover slip which was then inverted on the slide and the preparation sealed with vaseline.

The reticulocytes showed up quite as numerously as they did when brilliant cresyl blue was similarly applied, altho a little more slowly. In such preparations the reticulum appeared not infrequently in the form of filaments which usually had a tendency to interlace, sometimes quite densely so that there was a compact mass in the center of the red cell or perhaps eccentrically placed. At other times the interlacing network was more loosely formed and traversed the cell like a band. Most frequently the reticulum was observed to assume a different morphological character. This was a branching form which gave it an appearance not unlike moss. In such instances it was fuzzy and much less well defined. A reticulocyte might exhibit as little as a single small Y-shaped branch, or the branching forms might be scattered quite profusely thruout the cell. Regardless of what form the filamentous reticulum assumed, one or more metachromatic globules varying considerably in size and exhibiting Brownian movement were occasionally observed. In some instances these globules were isolated and at other times in intimate relationship with the filamentous material.

Dry slide preparations were made by spreading a moderately heavy film of the 0.3% dye solution on a glass slide and when this was dry, superimposing a blood smear thin enough so that the individual cells were separated. Wright's stain was then used as a counterstain in the usual way. In these preparations the reticulum usually appeared

[1]The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation, in coöperation with the Florida State Board of Health and the Florida State Hospital.

as granules of varying size and number. The intensity of the staining reaction varied greatly, as well as the amount of reticulum so precipitated. In some cells there was observed a light sprinkling of very fine granules, while others were packed with heavily-stained, short, thick rods. Still other reticulocytes might contain a relatively small number of larger, coarse granules which were frequently irregular in outline.

It appeared desirable to compare the efficiency of Wright's stain with that of the standard brilliant cresyl blue generally used as a reticulocyte stain. Parallel dry slide preparations with the two dyes were therefore made in the above-described manner, using blood from six patients who were convalescing from induced *vivax* malaria. Two thousand red cells were counted from each preparation and the number of reticulocytes was noted and the percentage determined. The results are summarized in Table 1.

TABLE 1. COMPARISON OF WRIGHT'S STAIN AND BRILLIANT CRESYL BLUE AS RETICULOCYTE STAINS

Case	Erythrocytes Millions	Hemoglobin Percentage	Erythrocytes counted*	Reticulocytes			
				Wright's		Brilliant Cresyl Blue	
				Number	Percentage	Number	Percentage
H. W.....	4.35	80	2000	168	8.4	142	7.1
O. W.....	3.08	58	2000	116	5.8	87	4.4
R. S.....	2.4	38	2000	122	6.1	116	5.8
L. P.....	2.04	38	2000	172	8.6	172	8.6
W. M.....	2.2	40	2000	139	7.0	107	5.4
T. D.....	3.68	70	2000	65	0.3	61	0.3
Total....	.....	.....	12000	782	6.5	685	5.7

*Number of erythrocytes counted in each Wright and brilliant cresyl blue preparation.

It will be seen that Wright's stain showed a definite advantage in four instances; that in one case there was no difference between the two dyes, and in another instance the difference, in favor of Wright's, was so slight as to be practically negligible. The total percentages indicate a significant advantage in favor of Wright's stain.

No attempt has been made to compare the reticulocyte staining

properties of samples of Wright's stain put out by different manufacturers. The only dye used in the investigation described above was that prepared by Dr. G. Grübler & Co., Leipzig. The writer has used this stain in another reticulocyte study,[2] the preparations for which had been made a year previously and had undergone no change.

The above data are presented because of the proven quality of Wright's stain and its usual availability in laboratories in which hematological work is done and because of the simplicity of its use in making satisfactory and durable reticulocyte preparations.

[2]Kitchen, S. F. 1938. The infection of reticulocytes by *Plasmodium vivax*. Amer. J. Trop. Med., (In press).

DIFFERENTIATION OF THE SECRETORY CELLS OF THE PARS NERVOSA OF THE HYPOPHYSIS CEREBRI

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ABSTRACT.—**A method was found by means of which two types of granular cells in the pars nervosa of the hypophysis cerebri could be preserved in permanent preparations so as to retain the appearance these cells presented in fresh tissue and in tissue cultures. The essential points of the technic were the fixation for 24 hours of the hypophysis in a solution composed of 2 parts of 3% potassium bichromate plus one part of a one-half saturated solution of bichloride of mercury in 95% alcohol. Sections of this material were prepared using dioxan in place of the higher alcohols and xylene. The sections were stained by means of Mallory's connective tissue stain leaving the sections in the fuchsin solution for 30 minutes and in the mixture of aniline blue, orange G and phosphotungstic acid for 1 to 24 hours.**

During the past few years many experiments have been carried out in this laboratory in an effort to obtain in permanent preparations the preservation of the cells of the hypophysis cerebri of different animals as they appear in fresh material and in tissue cultures.

In tissue cultures of the lobes of the hypophysis, especially of the posterior lobe, there occurred a multiplication and growth of more types of granular cells than were present in sections, even in those prepared according to newer methods, which show such beautiful granular cells in the pars anterior.

In preparations of the living pars anterior there were at least three distinct types of granular cells. One type had many comparatively large, uniform sized, brightly refractive granules that resembled those of an eosinophilic leucocyte, altho somewhat smaller; a second type had about the same number of granules of about the same size but less refractive, while the third type had many smaller granules that were not refractive. In the living pars intermedia the cells were full of characteristic granules, smaller than those in the cells of the pars anterior. In the living pars nervosa there were two types of granular cells. One type of granular cell, called the lipoglia cell by Gersh, contained globules composed mostly of fat, but the other type, which predominated, contained large and small granules composed of fat and protein, resembling the yolk granules found in many types of egg cells. In the glands from the animals studied groups of these granu-

lar cells were arranged along the blood vessels which ramify thru the pars nervosa.

Geiling and Lewis[1] found that the hormones characteristic of the posterior lobe of the hypophysis cerebri remained active in cells grown for many generations in tissue cultures. It, therefore, seemed of moment to describe the method that we found most favorable for the preservation in permanent preparations of the cells of the pars nervosa, as well as those of the pars intermedia and of the pars anterior as they appear in the living tissue.

Hypophyses of the mouse were used in the preliminary studies. Later the gland from the rat, rabbit, kitten, and chicken were fixed and stained by the method found to be most promising.

The difficulties encountered in preparing permanent preparations of the hypophysis were, first, the fixation of the different types of granules so that none of them became dissolved or distorted during embedding; second, the differential staining of the granules.

Glands were fixed according to 23 of the well-known methods of fixation and sections of each of these glands were stained by a number of the usual differential stains. Each of the methods of fixation gave excellent results in regard to one tissue or one type of granule, but only the alcoholic-chrom sublimate solution of Bensley and of Lane afforded uniformly good results in the preservation of the granular cells of the pars nervosa and the pars intermedia, as well as of the pars anterior.

This alcoholic-chrom sublimate solution contains one part of potassium bichromate (Bensley used a 2.5% and Lane a 3.5% solution)[2] added to one part of a saturated solution of mercuric chloride in 95% alcohol. After experimenting with modifications of this solution made up with 1, 2, 3 and 4 parts of the bichromate solution, added to one part of the bichloride solution, we found that one composed of 2 parts of a 3% solution of potassium bichromate and 1 part of a half saturated solution of mercuric chloride in 95% alcohol gave the most satisfactory results with the hypophyses of different animals. Corrossive sublimate is soluble in alcohol to about 33% and in water to about 7%; therefore, in order to avoid a heavy precipitate of bichloride crystals in the fixing solution, it was found advisable to use a half saturated alcoholic solution of mercuric chloride.

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[1]Geiling, E. M. K. and Lewis, M. R. 1935. Further information regarding the melanophore hormone of the hypophysis cerebri. Amer. J. Physiol., **113**, 534.

[2]Bensley, R. R. 1897. Mammalian gastric glands. Proc. Canadian Inst., **5**, 233.
————. 1901. The oesophageal glands of urodela. Biol. Bull., **2**, 87.
Lane, M. A. 1907. The cytological characters of the areas of Langerhaus. Amer. J. Anat., **7**, 409.

Most of the fixing solutions brought about considerable shrinkage of the gland. In the modified Lane's solution the shrinkage was slight and resulted in little distortion of the cells. The amount and rapidity of shrinkage, as also the length of time necessary for fixation, depended upon the size of the gland, whether it had been removed from the skull or whether it was protected with dura mater. Small glands removed from the skull were left in the fixing solution 4 to 6 hours and glands not removed from the skull were left 24 hours. The solution was changed once or twice during the first hour of fixation.

Sections of material fixed in this manner gave brilliant results with many of the differential stains. Preparations stained with Mallory's connective tissue stain, however, showed more types of granular cells than those stained by other methods. Mallory's stain gave almost as beautiful results on sections of the anterior lobe fixed by modified Lane's solution as on those fixed by the solutions recommended for that purpose. In preparations stained in this manner the pars anterior, containing many cells with brilliant red granules, some with blue granules and others with slightly yellowish granules, stood out sharply against the pars intermedia with its cells containing small, somewhat bronze granules. The pars intermedia was in turn clearly differentiated from the pars nervosa, composed of groups of cells containing blue to blue-bronze granules, arranged along the blood vessels between the unstained nerve fibers and non-granular neuroglia cells. This method did not preserve the fat globules, so that the lipoglia cells appeared as nuclei enclosed in a network of empty spaces.

For routine purposes we used the following technic.

Fixation—Modified Lane's Solution: 3% aqueous solution of potassium bichromate, 2 parts; one half saturated solution corrosive sublimate in 95% alcohol, 1 part.

1. Fix tissue 12–24 hours. One change of fixing fluid.
2. Transfer to 50% alcohol.
3. Transfer to 60% alcohol.
4. Transfer to 70% alcohol to which a few drops of iodin has been added. Change daily until the solution retains its color.
5. Dioxan. Three changes, each within 8 to 24 hours.
6. Dioxan to which a small amount of paraffin has been added. Paraffin is soluble in dioxan up to 5%.
7. Embed in paraffin using four changes, one-half to one hour in each; very small blocks about 15 minutes in each. Embed. Cut sections 5 μ thick. Mount on albuminized slides flooded with distilled water. Place on warm stage. When sections flatten out drain off excess of water. Arrange sections in order and allow to dry over night on warm stage.
8. Remove paraffin with xylene.
9. Absolute alcohol. Graded alcohols to water. Stain.

10. Stain sections in a 0.25% aqueous solution of acid fuchsin (Solution 1) for 30 minutes, then directly into:
11. Mallory's Stain (Solution 2) 1–24 hours:

Distilled water	100	cc.
Aniline blue	0.5	g.
Orange G	2.0	g.
Phosphotungstic acid	1.0	g.

12. Directly into 95% alcohol—several changes until no more color is given off.
13. Absolute alcohol. Xylene. Mount in balsam.

Prolonging the time the sections were allowed to remain in Solution 2 increased the amount of blue in the cells of the pars nervosa, but at the sacrifice of the brilliant fuchsin red in the cells of the pars anterior.

Leaving material in bichromate solution or mordanting sections in potassium bichromate decreased rather than improved the differential staining of the cells.

In order to attain the most satisfactory results with the method described we found it advisable to stain one section by the routine Mallory method, and, after examining it under the microscope, to correct the time the sections should be left in the fuchsin and in the Mallory stain, in order to suit the particular material or part of the hypophysis to be studied. The staining time varied with the species of animals. Kitten, mouse, rat and chicken hypophyses gave the best results when the sections were stained for 30 minutes in Solution 1 followed by one hour in Solution 2.[3]

Certain other stains gave excellent results with material fixed according to our method. Any stain containing fuchsin or phloxine in combination with methyl blue, methyl green or aniline blue differentiated two types of granular cells in the pars anterior. The fuchsin, picro-indigo carmine stain which gives such excellent results with the adrenal gland and with the ovary and oviduct, did not afford particularly good differentiation when used to stain sections of the hypophysis. Neutral gentian, a most desirable differential stain for the pancreas, stained the granules in the cells of the pars nervosa, so that the granular cells were sharply differentiated from the nerve fibers and non-granular cells; but the types of granular cell of the pars anterior were not differentially stained.

Conclusion: Fixation of the hypophysis cerebri in a modified Lane's solution, followed by Mallory's stain on thin sections, differentiated in permanent preparations the two types of granular cells in the pars nervosa, as well as the granular cells of pars intermedia and of the pars anterior as they appear in fresh material and in tissue cultures.

[3]Some sections of human and sheep hypophyses, fixed in 10% formalin, gave good results when left for 10 minutes in Solution 1 followed by 8 minutes in Solution 2

TECHNIC IN THE STUDY OF VASCULAR SYSTEMS IN PLANTS

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ABSTRACT.—**Methods have been improvised for studying the morphology of rosaceous fruits. One method is to dissect the buds and to immerse over night in 20% HCl; then in 5% NaOCl until bleached; after dehydrating, clearing and mounting in cedar oil, they are examined in dark field or with intense reflected light. Another method is to place the living stems, bearing buds, in a 0.01% aqueous solution of Niagara sky blue, forcing infiltration of the dye by means of pressure obtained in an ordinary pressure cooker thru the use of a bicycle pump; the buds are then dehydrated and embedded by ordinary methods. A series of photographs, if desired, may be taken of the cut surfaces of such a stained bud, after each section has been removed from the block.**

In the study of the morphology of pomes and other rosaceous fruits, it has been necessary to improvise methods in staining and cutting. As these may prove of value to other investigators following similar researches they are herein set forth.

As apples contain pectin, the lacto-phenol method of Simpson[1] proves unsatisfactory. Excellent results, however, are obtained by the following method: Buds are dissected and spread open to best expose the interior to view. This is done before bleaching because later they are too delicate to manipulate. They are then immersed over night in 20% hydrochloric acid to dissolve the crystals of calcium carbonate and oxalate. If the epidermis can be peeled off of the pubescent forms it should be done, as hairs beget artifacts and are generally troublesome. The buds are then placed in 5% sodium hypochlorite (commercial bleaching liquids are excellent and inexpensive). After being completely bleached (which is determined by examination with binocular) buds should be dehydrated by the alcohols in the usual way, then cleared in xylene, followed by cedar oil, and mounted *in toto* in either cedar oil or Canada balsam. Examined in a dark field, or by binocular with an intense reflected light, the vessels stand out very clear as white strands against a colorless background.

[1]Simpson, J. L. S. 1929. A short method of clearing plant tissues for anatomical studies. Stain Techn., 4, 131–2.

Another method is to place the living stems, bearing buds in an 0.01% aqueous solution of Niagara sky blue (Colour Index 520) until the color is seen in the petals. As this dye is water soluble but *not* alcohol soluble, buds stained in this manner do not destain when placed in 80% alcohol, and may thus be dehydrated and passed thru

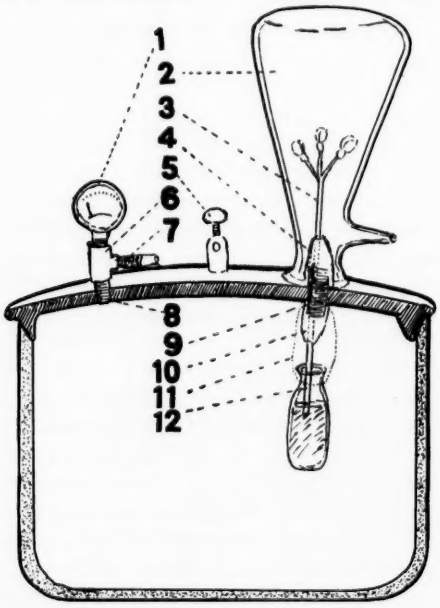

Fig. 1. Diagram of arrangements necessary to produce suction and pressure.

1. Pressure gauge.
2. Aspiration flask.
3. Stem bearing buds inserted thru rubber sleeves.
4. Rubber sleeve over nipple.
5. Exhaust valve.
6. 'T'.
7. Innertube valve stem.
8. Nipple.
9. Nipple, ends of which are covered with rubber sleeves 4 and 10.
10. Rubber sleeve over end of nipple 9.
11. Wire fastened about nipple to support bottle with dye.
12. Lower end of stem 3 inserted into dye in bottle.

xylene and paraffin or celloidin for sectioning. With this technic the vessels are a vivid blue and can be easily traced *in toto*, with clearing, or by sectioning.

To bring about a rapid infiltration of the dye the following apparatus has been improvised: An ordinary kitchen type pressure cooker is used, pressure employed to induce the dye to flow up the stem, and from above a vacuum produced to suck the dye up from below.

Most pressure cookers have standard ⅛ inch pipe thread fittings. The gauge and the safety valve are removed from the lid of the cooker and into the holes thus vacated, nipples are screwed. One nipple (length immaterial) has a 'T' screwed onto it and into one arm of the 'T' the gauge is screwed. Into the other arm of the 'T' the stem of an old-type inner tube value (whose base has been cut off with a hack saw) is screwed. This is made airtight by use of Dekotinski cement (the valve stem is also standard ⅛ inch thread, but being flattened on two sides it needs cement to seal). The second nipple should be about two inches long and should extend about ¾ inch above and also below the lid. A piece of 3/32 inch rubber tubing is placed over each end of this nipple and allowed to extend about ½ inch beyond

Fig. 2. Apparatus (using automobile pump) to produce pressure inside the chamber to force dye into stem, and suction (using Cenco vacuum pump) to suck dye into bundles.

the end. A wire is fastened about the nipple above the lower rubber tubing and from this a bottle containing the dye is suspended. A rubber stopper which snugly fits a wide-mouth aspiration flask, its hole cut large enough to fit about the nipple with its rubber sleeve, is cemented to the upper side of the lid.

A stem bearing buds is inserted thru the rubber sleeve from above and thru the rubber sleeve below, extending beyond into the bottle carrying the dye. The lid is now screwed tightly in place, the aspiration flask is placed over the buds, on the stopper, and its arm connected with an aspiration pump (or vacuum pump) which provides

the suction. The arm with the valve under the gauge is now connected with an ordinary automobile or bicycle foot pump which supplies the pressure from below. In a few minutes the blue color will be seen to appear in the petals having been drawn up thru *both* xylem and phloem.

By adopting the method of the workers of the Carnegie Institution doing embryological research at Johns Hopkins University,[2] a series of photographs can be obtained which are completely devoid of distortion. A camera is placed above the microtome block, which should be in focus and a photograph is taken of the block *after* each section has been cut. As every microtomist knows, sections are distorted by the knife, but with the photographic method, a perfect negative can be made of each section just *before* it is cut; these negatives projected upon cardboard or wax, enable one to make an exact model of the specimen, the blue dye clearly standing out for orientation of the enlargements so they may be correctly superimposed upon one another.

For the construction of the model of the vascular system, holes are cut or burned into the areas indicating the vessels; these holes should naturally register perfectly with those of the section below. Into the channels thus formed by the holes, short wires about ¼ inch in length are inserted, the wires first having been dipped into Dupont Duco Cement. These wires thus cemented to one another remain intact, and give a skeleton of the vascular system after the wax is melted away or the paper cut away.

Large paraffin sections readily break or curl, making it nearly impossible to obtain every section in a series. To obviate these difficulties a paper (or silk) apron[3] method has been found very satisfactory. This method is particularly applicable to the sliding microtome, altho it can be used with the rotary. It consists of using a piece of tissue paper or silk (a dark color preferred, to show the white paraffin by contrast) cut slightly narrower than the paraffin block but long enough to extend beyond the block on the side away from the knife edge. The apron, moistened in water, is placed upon the block so that the edge nearest the knife lies just within the edge of the paraffin. When the knife is run thru the embedded material, the section will be found adhering to the apron lying or *standing* on the edge of the blade. Flipped into the hand or removed with forceps directly, the section can be oriented on a slide (usual technic) and the apron

[2]Heard, O. O. 1931. A photographic method of orienting serial sections for reconstruction. Anat. Record, **49**, 59–69.

[3]Varrelman, F. A. 1931. The paper apron in microtomy. Science, **74**. 20.

removed and repeatedly used. This method prevents much distortion and shrinkage due to the fact that the section adhering to the apron by capillarity is held tightly in place and hence is not compressed and pushed together by the knife. Another valuable feature is that there is no danger of placing sections upside down, as the orientation is always the same. It is naturally far slower than the ribbon method, but no sections are lost, and curling is impossible. With this method it is possible to cut sections ½ μ thick. Naturally, a sharp knife is always necessary.

FANCY NAMES FOR STAINING SOLUTIONS

Some 40 years ago the well-known histologist, Arthur Meyer, began the practice of giving names to staining solutions. His *hemalum* and *carmalum* are so well known today that the names cause no confusion, and it is pretty well understood that they are solutions containing alum with hematein or carmine, respectively. Unfortunately the practice which Meyer began at that time has been followed in some quarters to such an extent as to cause considerable confusion at the present time.

The chairman of the Stain Commission is in a position to realize to some extent the confusion this practice of naming solutions can cause. Quite frequently some one of the stain companies writes in inquiring something as follows: "We have just received an order for the following but do not know of any dye of this name. Can you tell us what it could possibly be?" The name accompanying this question may be something like *azan carmine* or *ponceau fuchsin*, both of which terms were puzzling the first time we heard them. It frequently happens that some such name as this, which was coined in the first place for a mixture of stains or possibly for some particular staining schedule, is employed by some later author without any explanation or reference to show the reader what the solution or procedure actually was. It is quite natural, when the name thus coined sounds as much like the name of a dye as either of the two just mentioned, that a reader may well think it actually is a dye and may order it as such from a stain company. Since the stain companies seldom have the biological literature at their disposal, it is very difficult for them to know what the customer requires.

Especially unfortunate today is the practice of certain stain companies of putting out a considerable number of staining solutions under what one might well call fancy names, and keeping their formulae secret. The object of this, of course, is to increase the number of products they have for sale and to force users to describe the solution they employ by names that are meaningless except to the company by which they are manufactured. A few decades ago a solid dye could be put out under a new name and its nature kept secret; today there is little secrecy left in the dye business as such, so some of the stain manufacturers are trying to introduce secrecy by marketing solutions whose formulae are known only to themselves. The American stain companies have not done this to any appreciable

extent, but certain foreign concerns have long lists of such staining solutions and are doing their best to push their sales.

Users of biological stains will do much to help prevent this kind of confusion if they make it a rule never to coin a name for a staining solution and not to describe methods calling for named staining solutions unless they give the exact formula in their article so that there will be no misunderstanding on the part of readers. (There must, of course, be a few exceptions to this rule such as in the case of the well-known Wright stain and Giemsa stain, the general nature of whose formulae are very definitely known, altho each manufacturer may have his own special way of compounding the solution.) If this rule were followed generally it would make it necessary for companies selling such solutions to publish their formulae and would avoid considerable confusion on the part of those trying to follow the methods described. —H. J. Conn.

NOTES ON TECHNIC

PREPARATION OF LEUCO BASIC FUCHSIN FOR USE IN THE FEULGEN REACTION.—Scanlan and Melin[1] have drawn attention to the difficulty of obtaining a basic fuchsin suitable for the Feulgen stain and have described methods whereby the objectionable yellow to brown color, remaining in the fuchsin solution after bleaching with SO_2, can be removed. Their methods involve somewhat complicated processes of purification. I have recently obtained, thru the kindness of the I. G. Farbenindustrie A. G., Ludwigshafen am Rhein, directions for making the absolutely colorless standard solution of leuco crystal violet used in the "Ultraviolett-Dosimeter" designed by Weyde.[2] For the cytologist interested in the Feulgen stain the important feature is the use of a decolorizing carbon for removing the color remaining in the solution.

I have applied this method to the preparation of leuco basic fuchsin, using the decolorizing carbon Norit[3] in place of the animal charcoal recommended in the original directions, and find that a leuco fuchsin absolutely free from any trace of color can be easily prepared from samples of basic fuchsin which, as far as residual yellow to brown color indicates, represent very varying degrees of purity.

As the use of the carbon necessitates filtration and, further, as the finely divided carbon itself must carry into the solution a certain amount of oxygen, it is necessary to increase the concentration of SO_2 in the solution of basic fuchsin at the time of bleaching. In this way one can avoid the appearance of a faint pink tinge in the final product, indicating a slight oxidation of the fuchsin. This can be achieved by using double the amount of potassium metabisulfite recommended by de Tomasi.[4]

To 200 cc. of the solution of basic fuchsin, prepared in the ordinary way, are added 2 g. of potassium metabisulfite and 10 cc. of normal HCl. After allowing the solution to bleach for 24 hours, 0.5 g. of Norit

[1]Scanlan, J. T. and Melin, C. G. 1937. The production of basic fuchsin suitable for the Feulgen technic. Stain Techn., 12, 1–8.

[2]Weyde, E. 1933. Das Ultraviolett-Dosimeter, ein neues Instrument zur Messung ultravioletter Strahlung. Privatdruck I. G. Farbenindustrie.

[3]Norit is a proprietary vegetable carbon manufactured in several grades by the General Norit Company, Amsterdam, Holland. The American agent of the Company is L. A. Salomon, 216 Pearl Street, New York. The grade used by me is their supra neutral Norit.

[4]de Tomasi, J. A. 1936. Improving the technic of the Feulgen stain. Stain Techn., 11, 137–44.

is added. The whole is shaken for about a minute and is then rapidly filtered thru coarse filter paper. The result is a solution that cannot be distinguished from water in appearance. As stated, leuco basic fuchsin solutions prepared in this way from various sources and showing colors after bleaching ranging all the way from light straw color to brown have given Feulgen preparations which, for differentiation and colorless background, are all that could be desired.

I have used Norit, as it has been the decolorizing carbon available in this laboratory; but undoubtedly any one of the many other decolorizing carbons would serve equally well.—L. C. COLEMAN, Dept. of Botany, Univ. of Toronto, Toronto, Canada.

NOTE ON DIFFERENTIAL COUNTERSTAIN FOR THE GRAM TECHNIC.—Articles which were published[5] from the Bellevue-Yorkville Health Demonstration in New York City, contained descriptions of a new counterstain for Gram's technic, which consisted of a new type of methyl green and pyronin yellowish (National Aniline Co.). Investigation at this hospital since 1930 demonstrates the practical value of this procedure, both with direct smears and formalin fixed tissue. The method, first devised as an aid to gonorrheal diagnoses (along with cultures), was not intended to prove specificity of the gonococcus but rather to facilitate demonstration of the Neisserian group of cocci. The most recent batches of National Aniline methyl green (NG-10, 11 and 13) has greater dye content than other batches and the formula for the counterstain was subsequently adjusted as follows:

Special Gram Counterstain

Pyronin yellowish (Nat. Aniline)	0.1	g.
Methyl green (Nat. Aniline, NG-10 & 11 or 13)	0.65	g.
Hot distilled water	99	cc.

Let age for several days. Store in brown glass bottles in the dark.

This stain can be prepared in bulk and will keep for a long period of time. In addition to bacteria and ordinary cells, fungal mycelia and spermatozoa may be demonstrated. The head of the sperm cell is clear blue, unadulterated with red or purple.—SARA A. SCUDDER, Dept. of Pathology and Bacteriology, City Hospital, Welfare Island, New York, N. Y.

[5]Scudder, Sara A. 1930. Report to the Medical Committee, Milbank Memorial Fund, 325 E. 38th Street, New York City.

————. 1931. Differential stain favorable to the diagnosis of Neisserian infection. Stain Techn., **6**, 99–105.

————. 1933. Cervico-vaginitis of gonococcal origin in children. Hosp. Social Service Magazine, Supplement No. 1, March. p. 34.

Scudder, Sara A. and Lisa, J. R. 1931. Preliminary report on a combined Gram-Pappenheim stain for formalin fixed tissues. Stain Techn., **6**, 51–2.

LABORATORY HINTS

FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

The abstracts given here are intended primarily for laboratory use; consequently the technic in each instance is given in as much detail as possible.

J. A. de Tomasi — *Abstract Editor*

MICROSCOPE AND OTHER APPARATUS

JOHN, K. **Über einige neuere Hilfsgeräte für die Mikroskopie und ihre Anwendung in der Mikrophotographie.** *Zts. Wiss. Mikr.*, **54**, 414–9. 1938.

This paper is a commentary on the theoretical principles involved in the design and on the practical results obtainable especially in photomicrography with 3 new pieces of optical apparatus issued lately by Zeiss.

The pankratic condenser: This embodies the use of a combined light source and condenser. It carries also slits for filters, an aperture diaphragm connected with a scale, a pankratic optical system of 2 lenses, a mirror prism, and a common condenser. The beam of light travels horizontally to the prism where it turns vertically to illuminate the object as in all microscopes. This apparatus is fixed to the substage and offers 2 main advantages: The source of light never gets out of alignment, the figures and values on the diaphragm scale represent the apertures of the various objectives. The intensity of light was found sufficient for darkfield photography with pictures 9 x 12 cm. in size. (This apparatus is priced at about $125 in this country. *Ed. note.*)

The Mikropolychromar consists of a system of 2 light filter-condensers, possibly of complementary colors, which illuminate the object simultaneously. One of them has a numerical aperture smaller than that of the objective, and illuminates in its color the field of observation. The other's numerical aperture is bigger and illuminates in its color the object available in the field. As various combinations of colors are possible, it is also possible to choose that which will best interpret the results sought in terms of a record on a photographic plate.

Bernauer's polarization filter is made in the shape and size of the common ground glass disk that comes with the microscope. The polarization effect is due to a thin layer of "Herapathit" (a quinine-iodine compound) interposed between 2 polished glass surfaces. The shape and size of this filter is said to make it very useful in photomicrography, and the fact that it has a gray-greenish color protects it from excessive heating in artificial light.—*J. A. de Tomasi.*

KLEIN, B. M. **Das grosse Reichertsche Universalmikroskop "Z".** *Zts. Wiss. Mikr.*, **54**, 421–7. 1938.

In this detailed report all individual parts of Reichert's new massive Universal microscope "Z" are described and discussed at length. The revolving nose piece carries 4 objectives. Most of the parts, except the V base and the pillar, can be easily exchanged. This instrument can be made monocular, binocular, or provided with twin tubes and revolving pairs of objectives. A number of accessories include a sturdy metal bar intended to support the illuminating system and to keep it in line with the optical system.—*J. A. de Tomasi.*

PHOTOMICROGRAPHY

KRUSZYNSKI, J. **Vereinfachte Methode zur Bestimmung des Massstabes auf Mikrophotographien, besonders bei Anwendung eines dunklen Feldes oder des auffallenden Lichtes.** *Zts. Wiss. Mikr.*, **54**, 411–3. 1938.

A technic is suggested for superimposing a measuring scale upon photomicrographs. A number of pictures are taken, not larger than 4.5 x 6 cm., of an object micrometer with all the objectives that will be used in photomicrography. Positives are obtained by printing on thin film, or Gevaert Chromosa plates or Lumiere SE plates. If plates are used, the emulsion bearing the scale must be removed from the glass plate as follows: immerse 5–10 min. in 10% formalin, dry, and cut the emulsion pellicle along the edges. Immerse 5–10 min. in: 95% alcohol, 10 cc.; glycerin, 1 cc.; water, 90 cc. By means of tweezers, separate the film. Cut out a fragment of the scale and lay it upon a picture taken with the same objective.—*J. A. de Tomasi.*

MICROTECHNIC IN GENERAL

DAUKES, S. H. **New developments in visual teaching methods in use at the Wellcome Museum of Medical Science.** *J. Techn. Methods & Bul. Int. Assoc. Med. Museums*, **15**, 9–17. 1936.

A method of color preservation of fresh tissues is included. *Fixative:* Potassium acetate, 42.5 g.; KNO_3, 22.5 g.; water, 1600 cc.; formalin, 400 cc.; the salts are dissolved in a portion of the hot water and the remaining water and the formalin are added to the cooled solution. *Developer:* Fresh alcohol (i.e., strength not reduced by constant use). *Final fluid:* Sodium acetate 3,600 g.; dist. water, 12,000 cc.; 4% alc. camphor, 500 cc.; glycerin 7,200 cc. Add in the order given. At first some of the camphor is thrown out of solution but it is redissolved on standing. The length of time for each step is not given.—*M. Lois Calhoun.*

HERRATH, E. v. **Ein einfaches Verfahren, dicke Zelloidin-Paraffinschnitte zu strecken und aufzukleben.** *Zts. Wiss. Mikr.*, **54**, 413–4. 1938.

The celloidin-paraffin method, altho one of the most reliable, still offers occasions for improvements. Above all, thick sections very often do not spread satisfactorily nor cling to the glass as desired. A technic intended to correct this defect is suggested: Unroll curled sections with 2 brushes on a glass slip smeared with albumin-glycerin and covered with dist. water. Hold down sections with a strip of coated paper as big as the slide. Press down with the thumb and wet again with dist. water. Place in a 40–45° incubator for 24 hr. to loosen the paper. Coagulate the fixative carefully by holding over a flame.—*J. A. de Tomasi.*

LEVI, G. **Eine neue Methode zur Befestigung der Zelloidin-Serienschnitte an Gläsern.** *Zts. Wiss. Mikr.*, **54**, 406–8. 1938.

Serial sections of celloidin embedded material are affixed to the slide or cover glass as follows: Arrange the sections on the glass in the order desired so that their edges touch. Keep them wet with 96% alcohol. Cover with a square of clean paper and press down quickly. The sections will stick to the glass surface and the excess alcohol will be absorbed by the paper. Remove the paper and before the slide dries pass it to a 1:4 mixture of abs. alcohol and ether; time varies between 10–20 sec. for large to 1 sec. for very small sections. Transfer to 70% alcohol to reharden the celloidin, then to water. Stain and mount as usual. This technic has proved satisfactory with a number of the most common fixatives and stains.—*J. A. de Tomasi.*

MAGRUDER, S. R. **A comparative study of dehydration.** *J. Lab. & Clin. Med.*, **23**, 405–11. 1938.

Acetone and dioxan (diethylene dioxide) are the best dehydrating agents. Cellosolve and tertiary butyl alcohol in most cases gave very poor results being used undiluted and not in a graded series as recommended by Frost and by Johansen. All work with dioxan should be done in a chemical hood because inhaling even a small amount of dioxan fumes causes serious bronchial, liver and kidney disturbances.

Technic: Fix in hot formalin for 5 min. Wash in running water 10 min. Dehydrate in 2 changes of undiluted dehydrating agent for 1 to 1½ hr. Embed

in paraffin. Section. Remove paraffin in xylene. Dehydrate without diluting. Pass to dist. water, stain in hematoxylin, and tone in alkaline tap water. Rinse in dist. water and counterstain in eosin dissolved in dehydrating agent. Wash in dehydrating agent. Mount in Canada balsam. Total time required, about 2 hr.—*A. V. Tolstoouhov.*

SAUER, F. C. **A method of making stereoscopic illustrations.** *J. Optical Soc. Amer.*, **27**, 350–4. 1937.

This method, devised by a zoologist, is reported here because it suggests a good way of representing graphically the internal structural location of the parts of an object, as in the case of a cell. Being a modification of current procedures based on drawings, it aims to produce a perspective picture of the object from tridimensional measurements between some of its internal points. By means of a ruled transparent sheet, a mirror image or "stereoscopic mate" is developed so that all points are automatically placed. Looking at the 2 drawings thru a stereoscope, the tridimensional effect is quite evident. The duplicate figure cannot be so easily obtained by any other graphical means. Stereoscopic illustrations are thus produced at only slightly higher labor cost than ordinary drawings. —*J. A. de Tomasi.*

TERNI, T. and DE LUCCHI, G. **Sulla istocarbonizzazione (istopirolisi): Primi risultati per cellule spermatiche.** *Bull. d'Histol. Appl.*, **14**, 209–22. 1937.

This paper is a preliminary report on studies upon a new departure in histochemical methods. It concerns itself with the changes produced in sections and smears by temperatures ranging around 350° C., and the development of brown colorations which might be used in locating specific organic compounds. Isolated cellular elements, including spermatozoa, blood cells, and bacteria are used. The treatment, called histopyrolysis, a partial carbonization, is carried on under the microscope by placing the slide upon a Klein's disc (Reichert, Vienna) containing the heating unit, a resistance attached to a rheostat. This heating plate has a hole in the middle to allow illumination of the preparation. A condenser is inserted at its upper opening and the lens of the objectives have all-metal mountings. The temperatures, checked by a suitable thermometer, range between 30° and 350° C. with a possible increase of 10° per minute. The technic consists in heating slowly up to 100°, then up to 340–350° C. in about 20 min. Interesting changes of shape and color of cell structures take place which can lead to further appreciation of cytological details. In the case of spermatozoa, results may point to new views on the part lipids play in the constitution of the acroblast and the cephalic cap.—*J. A. de Tomasi.*

ZETHRAEUS, S. **Modifikation der Schultz-Braunsschen Gefrierschnittmethode.** *Zts. Wiss. Mikr.*, **54**, 408–11. 1938.

In the past cooling of the knife blade on freezing microtomes could only be obtained by applying a special arrangement whereby part of the CO_2 stream also cooled the blade. The operation of more than one stopcock and the stirring of warm air currents proved to be serious defects. The method here proposed simplifies the manipulations and eliminates the former objections.

Instead of using compressed CO_2, brought to it from a tank, the blade carries a brass box shaped to fit it tightly which can be filled with a mixture of ether and CO_2 snow; 100 cc. of it will keep the blade frosted for about 20 min., which is usually sufficient for most histological sectioning. More prolonged action is obtained when dry ice is added instead of CO_2 snow.—*J. A. de Tomasi.*

DYES AND THEIR BIOLOGICAL USES

JOYNER, A. L. and SABIN, F. R. **Differences in spread of dye in skin of normal and tuberculous guinea pigs.** *Proc. Soc. Exp. Biol. & Med.*, **37**, 373–5. 1937.

The dye used, pontamine sky blue, in an approximately isotonic 2.5% solution, was injected intradermally in 0.025 cc. doses in tuberculous and non-tuberculous guinea pigs. Diffusion was more rapid and more extensive in normal animals, except that in the terminal stages of infection tuberculin-negative animals react like normal animals.—*M. S. Marshall.*

KEMPTON, R. T., BOTT, P. A. and RICHARDS, A. N. **The glomerular elimination of indigo carmine in rabbits.** *Amer. J. Anat.*, **61**, 505–21. 1937.

When by ureteral injection, the lumina of tubules and Bowman's capsules of frogs' kidneys were filled with known solutions of indigo carmine, sections of the tissues, fixed and mounted according to the technic of Holton and Bensley, contained recognizable residues of dye in those spaces only when the injected solution contained more than 8 mg.%. Sections of the kidneys of rabbits which had been injected intravenously with indigo carmine showed residues of dye in the intracapsular spaces only when the dosage of dye was such that an ultrafiltrate of plasma taken at the time of excision of the kidneys contained more than 8 to 10 mg.%. The fact that other investigators, in similar experiments, have failed to detect it in Bowman's capsules and have, in consequence, maintained that indigo carmine is not excreted thru the glomeruli appears to be due to the rapidity with which the concentration of dye in the plasma diminishes after its injection and to the large percentage which is bound to the plasma proteins and is therefore nonfiltrable. Calculations of clearance, however, indicate that the fraction of dye excreted thru the glomeruli is small; that excreted by the tubules, large.—*Authors' Abstract.*

McCRADY, M. H. **A practical study of procedures for the detection of the presence of coliform organisms in water.** *Amer. J. Pub. Health*, **27**, 1243–58. 1937.

With 21 laboratories in the United States and Canada collaborating, a total of 1231 samples of water were examined. Lactose broth presumptives were obtained from them, employing 5 different methods of confirmation: the "completed test" of *Standard Methods of Water Analysis*, and 4 liquid media methods employing, respectively, 2% brilliant green bile, crystal violet, fuchsin, and formate ricinoleate broths. The results indicated the brilliant green bile confirmatory method to be the most generally satisfactory of the selective procedures. The results also indicated that the brilliant green bile method compared very favorably with the usual *Standard Methods* "completed test" and, for the examination of most waters, might advantageously replace it. For finished waters, the alternative "completed test" of *Standard Methods* is suggested.—*M. W. Jennison.*

McMASTER, P. D. and PARSONS, R. J. **Path of escape of vital dyes from the lymphatics into the tissues.** *Proc. Soc. Exp. Biol. & Med.*, **37**, 707–9. 1938.

Pontamine sky blue and patent blue V, of widely different diffusibility, were introduced directly into the lymphatics of the ears of living mice, and the course of the dye was followed at high magnification thru the Ultropak microscope. Fine wavy lines, which could be moved intact, invariably appeared, apparently indicating movement of the dyes along tracks determined by the form and situation of connective tissue fibers. In the presence of edema the fluid itself became stained. Either 4% brom cresol purple or 4% brom thymol blue produced edema rapidly, but this was preceded by the appearance of sharp colored lines.—*M. S. Marshall.*

OSTROUMOV, V. G. **The action of ultra-violet rays on the peroxidase granulation of leucocytes.** *Arch. Russ. Anat. Histol. Embr.*, **16**, 89–96. 1937.

This paper gives an account of cytological observations carried out on blood cells exposed to solar and artificial ultraviolet irradiation. Dry blood smears are fixed in alcohol-formalin, treated in a H_2O_2–α-naphthol solution and stained with Epstein's toluidine blue solution. The peroxidase reaction consists in a green color developing in certain cellular granules thus treated. The smears are then exposed to the ultraviolet irradiation. It is found that the peroxidase reaction is destroyed by light of 300–312 mμ wave length; that eosinophils show greater resistance to discoloration than neutrophils; that fat solvents also destroy the chromatic properties of the granules. After irradiation these change from spherical to an irregular shape. It is inferred that the seat of the peroxidase chromatic reactions is the lipoid surface layer of the granules, which is likely to act as the carrier for the enzyme.—*J. A. de Tomasi.*

POTTS, A. M. and KOCH, F. C. **A new apparatus and an improved method for chromatographic adsorption.** *Proc. Soc. Exp. Biol. & Med.*, **37**, 300–3. 1937.

The apparatus, shown by a diagram, differs from the older forms in use for the separation of organic compounds in that it filters the solution thru the column of adsorbent by pressure and not by suction. The area of specific adsorption of colorless compounds is indicated by a color reaction carried out on one side of the column of adsorbent or can be located by the fluorescence in ultraviolet light.—*M. S. Marshall.*

ROSENBLUM, L. A., HOSKWITH, B. and KRAMER, S. D. **Photodynamic action of methylene blue on poliomyelitis virus.** *Proc. Soc. Exp. Biol. & Med.*, **37**, 166–8. 1937.

Concentrations of 1:25,000–1:50,000 of Grübler's methylene blue "for bacilli, *Koch*", in saline, autoclaved, were mixed with equal parts of a suspension of poliomyelitis virus. They were exposed in a thin layer to a new 100-watt bulb at 20 cm. distance, for 30–60 min. Inactivation of the virus was most regular with a 1:100,000 final dilution of the dye after exposure for 60 min.—*M. S. Marshall.*

SCHOUR, I. and HOFFMAN, M. M. **Effects of alizarin red S injections on the teeth and bones of Macacus rhesus monkey.** *Proc. Soc. Exp. Boil. & Med.*, **37**, 710–1. 1938.

Multiple intravenous, intraperitoneal or subcutaneous injections were given, using 5–10 cc. of 2% alizarin red S (sodium sulphalizarate, C. I. No. 1034, Coleman & Bell) at 5–30 day intervals. Results were obtained between 3 hr. and 125 days. A permanent red ring appeared in dentin and bone, calcifying at the time of injection. The use of sterile 2% dye altered with isotonic NaOH, given intraperitoneally, is suggested for sharp effects with little toxicity.—*M. S. Marshall.*

T'UNG, T. **In vitro photodynamic action of methylene blue on Trypanosoma brucei.** *Proc. Soc. Exp. Biol. & Med.*, **38**, 29–31. 1938.

Citrated blood of infected guinea pigs was lightly centrifuged to throw down blood cells. Nine parts of supernatant fluid, containing organisms, were mixed with 1 part of various dilutions of methylene blue. The suspensions were exposed over a cooling unit to a 100-Watt lamp at 10 cm. distance for 30 and 60 min. The exposure of trypanosomes, for either 30 or 60 min., to dilutions of methylene blue up to 1:100,000 immobilized the organisms and destroyed their lethal activity. Without exposure to light a 1:100 concentration of dye was required to produce a similar effect.—*M S. Marshall.*

ANIMAL MICROTECHNIC

DEADMAN, J. and ANSLEY, H. A. **Preparation of a series of brain tumors.** *J. Techn. Methods & Bull. Int. Assoc. Med. Museums*, **15**, 36–8. 1936.

The principle involved is that the myelinated portion of nervous tissue will stain blue with $K_4Fe(CN)_6$, while the gray matter remains unstained. The following procedure is recommended: Fix in formalin. Wash in running water and finally in dist. water. Immerse for 2 min. at 60–65° C. in a solution of 4% phenol, 0.5% $CuSO_4$, and 0.125% HCl. Wash. Immerse 2 min. in 1% $FeCl_3$. Wash 5 min. in running water. Immerse in 1% $K_4Fe(CN)_6$ until a brilliant blue color develops. Wash and mount in 70% alcohol or Klotz fluid. This is particularly useful for photography.—*M. Lois Calhoun.*

DE GALANTHA, E. **Improved method for rapid decalcification.** *J. Techn. Methods & Bull. Int. Assoc. Med. Museums*, **17**, 72–3. 1937.

The following procedure is suggested as a method for improving the usual decalcification technic. Fix in 10% formalin 24 hr. Cut blocks of bony tissue as thin as possible and place in the following mixture: Add 20 cc. of conc. HNO_3 to 50 cc. of dist. water. Add slowly to 200 cc. of 95% ethyl alcohol. (*Caution*—use cold solutions.) Add 10 cc. of conc. picric acid (concentration not given). Add 10 cc. of pure olive oil and mix thoroly. After 24 hr. begin testing the tissue with a fine needle to determine the extent of decalcification. When the consistency is that of soft tissue, wash thoroly in running tap water for 12 hr. Place

blocks in 2% ammonia solution for 24 hr. Wash in running water for 12 hr. Dehydrate, embed in paraffin, cut, and stain.—*M. Lois Calhoun.*

ENDTZ, A. W. **A method to stain blood cells directly in undried urine sediments.** *Münch. med. Woch.*, **85**, 141. 1938.

To a drop of centrifuged sediment on a slide add a drop of a reagent prepared by mixing 1% alc. ("spiritus dilutus") benzidine solution (Sol. I), 1 ml.; 1% aq. acetic acid (Sol. II), 1 ml.; 3% H_2O_2, 3 drops; and dist. water, 9 ml. Mix well and examine under cover glass. The erythrocytes are uniformly stained deep blue, while the other blood cells exhibit structural elements. Cellular elements not of blood origin remain unstained. To differentiate the various white blood cells, a reagent composed of Sol. I, 0.5 ml.; 3% H_2O_2, 3 drops; dist. water, 5 ml.; and Sol. II, 5.5 ml. is used. This reagent gives the best differentiation between erythrocytes, lymphocytes and leucocytes, whereas the first reagent brings out the erythrocytes better. The reagents can be kept for at least 2 weeks.—*L. Farber.*

EVANS, F. L. **A timesaving method for examining blood films with the high dry (45×) and oil immersion objectives.** *J. Lab. & Clin. Med.*, **23**, 646. 1938.

The advantage of the method is that a larger surface of the slide can be examined and in less time than with the oil immersion method. The effect is as brilliant as tho the smears were permanently mounted under cover glass. Spread a drop of immersion oil evenly over the entire slide with a piece of glass rod or tubing and let it stand for a few minutes. The film should be thin enough to permit focusing without touching the oil.—*A. V. Tolstoouhov.*

GOLDNER, J. **A modification of the Masson trichrome technique for routine laboratory purposes.** *Amer. J. Path.*, **14**, 237–43. 1938.

Paraffin sections of material fixed in Bouin's or Zenker's fluid stain best, altho formalin fixation can be used. Remove paraffin and pass thru graded alcohols to lithium carbonate solution after Bouin's, or thru I_2 and $Na_2S_2O_3$ after Zenker's; no special treatment after formalin. Wash. Transfer from water to either Hansen's or Weigert's iron hematoxylin for 1–5 min. Wash in tap water and differentiate with dilute acid (aqueous or alcoholic) if necessary to bring out the nuclei. Stain 5 min. or more in Masson's fuchsin-ponceau (ponceau de xylidine, Krall or Hollborn, 0.2 g.; acid fuchsin, any good brand, 0.1 g.; dist. water containing 0.2% acetic acid, 300 cc.). Rinse in 0.2% acetic acid. Treat 15 sec. to 30 min. with: phosphotungstic acid, 3–5 g.; orange G (Hollborn standardized), 2 g.; dist. water, 100 cc.; a few minutes are usually sufficient. Repeat the rinse in 0.2% acetic. Stain 5 min. in Masson's light green (light green 0.1–0.2 g.; 0.2% acetic acid, 100 cc.). Rinse in 0.2% acetic acid to eliminate the phosphotungstic acid and differentiate the colors. Dehydrate and mount in balsam. Azophloxine may be added (in a final concentration of 0.01%) to the fuchsin-ponceau solution if desired.—*H. A. Davenport.*

GROSSFELD, H. **Osmotische Druck und Vitalfärbung.** *Protoplasma*, **29**, 261–71. 1937.

Chick or rat embryo tissues are immersed in hanging drops of a mixture of embryonic extract, Ringer's or Tyrode's solution, and dist. water (5:4:1). In this hypotonic medium, growth of fibroblasts or epithelium takes place rapidly. The medium is then removed as much as possible and replaced by a solution of neutral red in Ringer's fluid. If the Ringer's solution is thus diluted more than 3–5 times, the dye stains the cytoplasm and nucleus diffusely instead of giving the usual granular cytoplasmic stain. The shift from diffuse to granular staining is reversible by changing the medium. Changes in H-ion concentration have no effect.—*R. Chambers.*

HADEN, R. L. **Diffraction methods for measuring the diameter of the red blood cell.** *J. Lab. & Clin. Med.* **23**, 508–18. 1938.

Measuring the size of the erythrocyte is a valuable procedure in the study of blood dyscrasias. Diffraction methods are satisfactory for the purpose. The Emmons eriometer has the advangate of portability and does not require a dark room. (The Emmons eriometer is not now available on the market.) Pijper's

blood-cell-tester and Emmons' eriometer give accurate results in every case. Bock's erythrocytometer readings tend to be too low if the cells are large, and too high if the cells are small. The readings with the Emmons and Bock instruments are the same for stained as unstained films. The Pijper blood-cell-tester is excellent for comparing an abnormal with a normal film and also indicates the degree of anisocytosis as well as the mean diameter. With the Pijper instrument the diameter measures about 0.5 mm. less on a stained than on an unstained film. —*A. V. Tolstoouhov.*

HALPERN, S. R. **Quantitative cytological studies of the anterior lobe of the hypophysis of fetuses and children, correlated with sexual and skeletal development.** *Endocrinology,* **22,** 173. 1938.

The author describes a modification of Martin's differential stain for tissue. Sections are treated as follows: 0.5% aq. erythrosin, 30 sec.; drain excess stain and pass directly into 1% aq. phosphomolybdic acid, 90 sec.; drain and pass into 0.5% aq. methyl blue, 50–60 sec.; differentiate in 95% alcohol; pass thru abs. alcohol and xylene; mount in balsam.—*L. Farber.*

JALOWY, B. **Kollagen, Elastin, und Retikulin der Haut.** *Zts. Zellforsch. Mikr. Anat.,* **27,** 667–90. 1937.

Reticulin fibers can be easily differentiated from collagen by modifying the method of Hortega as follows: Fix 24–48 hr. in neutral formalin, 1:4; embed in paraffin; section; remove paraffin and hydrate. Stain at 30°C. for 5–30 min. or longer in following ammoniacal silver solution: 10% $AgNO_3$, 20 cc.; 40% NaOH, 20 drops; wash precipitate 10 times in dist. water; suspend in 20 cc. dist. water; add NH_4OH (sp. gr. 0.879–0.910) by drops and stir until ppt. begins to dissolve; add 20 cc. dist. water and enough NH_4OH to dissolve ppt.; centrifuge if necessary, add 100 cc. of dist. water to the supernatant solution and store in the dark until needed. Rinse in dist. water, and in ammonia water (10 drops NH_4OH to 100 cc. dist. water) to remove the loose silver adhering to the collagen. Place in neutral formalin (1:4) for a very short time; wash in running dist. water; dehydrate, and mount in balsam. The argentophilic reticulin fibers and their terminal branches are impregnated dark with silver; the unchanged collagen is yellow or brownish yellow; cells and their nuclei are clear yellow or unstained.—*V. Warbritton.*

KREMER, J. **Eine einfache und empfehlenswerte Methylgründoppelfärbung des Kaltblüterpankreas mit besonderer Hervorhebung der Zymogengranula.** *Zts. Wiss. Mikr.,* **54,** 419–21. 1938.

A case is reported where a single dye (methyl green) has given a two-color differential stain. Frog pancreas is fixed in Zenker's, washed, treated in I–KI alcohol, embedded and cut as usual. The sections, after spreading and decoration, are deiodized in $Na_2S_2O_3$ and treated for 12 hr. in 4% Fe alum. Dyes used are principally methyl green N and methyl green pure (Grübler). The staining consists of a simple immersion for 12 hr. in: methyl green, 1 g.; phenol, 0.25 g.; dist. water, 100 cc.; then rinse, blot, dehydrate and mount. Particularly striking is the contrast between the blue to red-violet zymogen granules and the plasma structures, nuclei and nucleoli, which stain green. This is evidently due to the methyl violet which is a frequent impurity in the green homologue.—*J. A. de Tomasi.*

LAWSON, W. H. **Elastic tissue staining.** *J. Techn. Methods & Bull. Int. Assoc. Med. Museums,* 16, 42–4. 1936.

This technic is a combination of the familiar Weigert's method and Sheridan's modification.

Solution 1: Gentian violet (British Drug Houses), 2 g.; resorcin, 4 g.; tap water, 100 cc. Dissolve and boil 3 min. Stir, and add 16 cc. of Liq. ferri sesquichlor; a German preparation (P.G. III) is said to be preferable to ordinary $FeCl_3$. Boil again and add basic fuchsin (Gurr) 2 g.; tap water, 50 cc.; Liq. ferri sesquichlor, 15 cc. Boil and filter.

Solution 2: Boil the filtered deposit and the filter paper in 150 cc. of 95% alcohol for 3 min. Filter and bring the filtrate to 150 cc. with 95% alcohol. Add 4.5 cc. of HCl.

To Sol. 1 add Sol. 2, including the filter paper and any deposit on it. Bring to a boil. Add 4 g. of resorcin and continue to boil for a few minutes. Filter. The

filtrate is the final staining solution and the acid content may be increased to 3% if desired. Stain 30–60 min. Differentiate in abs. alcohol, or if necessary, in 2% acid alcohol.—*M. Lois Calhoun.*

MATHIS, J. **Schnittfärbung mit Hämalaun-Erythrosin-Orange.** *Zts. Wiss. Mikr.*, **54,** 428–9. 1938.

A better differentiation and therefore more contrast between hematoxylin and eosin is often desired. The following technic worked satisfactorily with various kinds of animal tissues and cells, including blood leucocytes: Fix in any of the common solutions (Bouin, Zenker, Orth, Helly, Maximov, Heidenhain's "Susa", Schaffer). Stain intensely in Mayer's acid hemalum. Wash thoroly in tap water. Dip in 70% alcohol. Overstain considerably with erythrosin or eosin. Transfer immediately to orange G solution (sat. aq. orange G, 10 drops; 96% alcohol, 10 cc.). Watch under the microscope the progress of differentiation (usually ½ min.). Rinse shortly in 96% alcohol and flatten out with a brush moistened by the alcohol. Blot with filter paper. Clear and mount.—*J. A. de Tomasi.*

MATTOX, N. T. **Oogenesis of Campeloma rufum, a parthenogenetic snail.** *Zts. Zellforsch. Mikr. Anat.*, **27,** 455–64. 1937.

A fixative consisting of 85 parts 70% alcohol, 10 parts 40% formalin, and 5 parts glacial acetic acid is used, apparently in the same way as Bouin's fluid. No comment is made on its value.—*V. Warbritton.*

MEEKER, L. H. **Gelatin embedding for rapid frozen sections of "curettings".** *J. Techn. Methods & Bull. Int. Assoc. Med. Museums*, **16,** 41–2. 1936.

A rapid gelatin method is given which is adapted to frozen sectioning of fragments of tissue. *Sol. 1*—(Jelly): 30% gelatin; 5% phenol; put in 5 cc. quantities in small bottles. *Sol. 2*: 50% formalin; put 5 cc. quantities in small bottles. Store both at 8° C. Heat to 60° C. in a water bath when ready for use.

Procedure: Wrap the curettings in lens paper, place in 20% formalin and heat to boiling 1 min. Place rubber ring (section of rubber tubing 20 mm. in diameter) on microtome stage, coat ring on the inside with vaseline, and fill with the fixed curettings. Mix Sols. 1 and 2 with a warm glass rod, pour over curettings, freeze. The formalin and jelly-mixture sets instantaneously and binds the tissue fragments firmly. Remove rubber ring and float sections on ice water. Mount on slide. The sections will adhere firmly. Stain in dilute thionin (concentration not given) and mount in glycerin. Time required, 5 min.—*M. Lois Calhoun.*

SANCHEZ-PEREZ, J. M. **Hortega's silver stains in neuropathology.** *J. Techn. Methods and Bull. Int. Assoc. Med. Museums*, **15,** 74–83. 1936.

This is a summary of the variations of Hortega's methods and includes detailed procedures for 24 modifications. The following gelatin embedding technic for tissues too friable to section with a freezing microtome is given: Wash tissues in water 24 hr. Infiltrate 24–48 hr. at 36° C. with weak solution of gelatin (1 part, by volume, of granular gelatin to 1 part of 5% phenol, subsequently diluted with twice its volume of water and held at 36° C.). Continue infiltration at 36° C. for 12–24 hr. in conc. gelatin (equal volumes of gelatin and 5% phenol). Remove from incubator and cool, cut out embedded blocks and harden 24 hr. in 10% formalin. Section with freezing microtome. Stain in the usual way. In mounting, put 8 drops of creosote and 2 drops of ammonia (conc.?) on the section before adding alcohol. Agitate well before pouring it off. Add alcohol and clear in creosote.—*M. Lois Calhoun.*

SCHLEIFSTEIN, J. **A rapid method for demonstrating Negri bodies in tissue sections.** *Amer. J. Pub. Health*, **27,** 1283–5. 1937.

Blocks of not more than 3 mm. thickness of fresh tissue from the hippocampus major and cerebellum are fixed in Zenker's solution, washed, dehydrated in dioxan, embedded in paraffin, cut not more than 4μ in thickness, and the paraffin removed. Mount on slides. A modified Wilhite stain is used.

Stock solution: Rosanilin (Grübler), 1.80 g.; methylene blue (Nat. col.), 1 g.; glycerol 100 cc.; methyl alcohol 100 cc. This keeps indefinitely.

For staining, add 1 drop of a 1:40,000 aq. sol. of KOH to 2 cc. of stock solution. This mixture should be freshly prepared each time. Flood slides with stain and

steam gently for 5 min. Cool and wash quickly in tap water. Decolorize and differentiate each section separately by gently swishing the slide in a jar of 90% ethyl alcohol until the section assumes a faint violet color. Pass the section rapidly thru 95% alcohol, abs. alcohol and xylene. Mount in Canada balsam. Negri bodies stain a deep magenta red and, as seen under high magnification, the granular inclusions are dark blue. Nucleoli appear bluish-black; cytoplasm, bluish violet; and red blood cells, a copper color.—*M. W. Jennison.*

v. HAAM, E. and ALEXANDER, H. G. **A new method of vital staining of the nucleus and nucelolus in cell suspensions and frozen sections.** *J. Techn. Methods and Bull. Int. Assoc. Med. Museums,* **15,** 70–3. 1936.

A new method is recommended for the vital staining of nuclei and nuceoli in cell suspensions or frozen sections.

Cell suspensions: Add a 1% aq. sol. of toluidine blue to a well shaken preparation of cell suspensions from fresh tissue, shake again and centrifuge. Make smears on slides and examine wet or dry. Protoplasm is practically unstained, nucleus a faint blue color, nucleolus a deep purple red. The smears may be covered with shellac and kept several weeks. For photomicrography, counterstain the dry smears with a 1% aq. sol. of methyl green for increased contrast.

Frozen sections: Cut sections of fresh tissue 10 μ or less and place in 10 cc. of dist. water to which has been added 0.1 cc. of a 1% aq. sol. of toluidine blue (1:10,000 dilution). Leave in this until the sections take on a faint bluish color, wash in dist. water and mount.—*M. Lois Calhoun.*

PLANT MICROTECHNIC

WEISE, R. **Über die Brauchbarkeit von Trypanblau zur mikroskopischen Färbetechnik.** *Zts. Wiss. Mikr.,* **54,** 398–405. 1938.

Trypan blue (Grübler) is a suitable microchemical stain for plant tissues.

Permanent mounts: Dilute 0.1% aq. trypan blue in 96% alcohol so as to obtain an 80% or even a 70% alc. solution of the dye; filter. Stain immediately after the abs. alcohol. There is no danger of overstaining and practically no dye is lost in the dehydration, or in the xylene used in the Canada balsam mount (exact time not given).

Results: Cellulose walls are stained light blue to dark violet; walls with reserve cellulose (hemicellulose) colorless to weakly stained; cutin and suberin impregnations, unstained; Casparian strips of endodermis, unstained; lignified membranes, light blue, sometimes dark blue, never violet; middle lamellae of lignified tissues, dark violet. Cell contents are stained: mucilage (in some plants), blue; starch, unstained; aleuron granules: crystalloid, strongly red violet, and globoid, unstained; nucleus, never blue, always reddish violet or violet blue. Slightly varying shades are obtained due to the presence of a red and a blue component in the dye. The factors governing these variations have been determined.

Temporary hand sections: Treat sections for a short time in 0.1% aq. sol. of trypan blue. Rinse with water.—*Thora M. Plitt.*

MICROÖRGANISMS

DIENST, R. B. **Further observations on the use of nigrosine to demonstrate Treponema pallidum in syphilitic lesions.** *J. Lab. & Clin. Med.,* **23,** 646–8. 1938.

Nigrosin is far superior to any other agent used for this purpose. It gives the same percentage of positive findings as dark field illumination.

Stain: Prepare 5% aq. solution of nigrosin (Coleman & Bell) by heating either on a water bath for 30 min. or in an autoclave at 15 lb. for 15 min. Add 0.5 cc. 37% formalin per 100 cc. of stain as a preservative. Filter just before using.

Method: On a clean slide, place 1 small loopful of exudate from a cleansed lesion into a loopful of water. Add a loopful 5% nigrosin solution, mix rapidly by rotary movements, gradually increasing the size of the smear. Dry. Place a drop of immersion oil on the smear and examine with good substage lamp. *Treponema pallidum* appear as finely coiled, colorless organisms. The background is stained gray to blue black, depending on the thickness of the smear. Focus sharply. Sometimes the delicate terminal filaments can be seen. Satisfactory results have been obtained by using exudate dried first on the slide. In this case a loopful of water is placed on the dried exudate. Mix a loopful of the dissolved exudate with

a loopful of nigrosin solution, smear, dry and examine as fresh exudate.—*A. V. Tolstoouhov.*

FULLER, D. S. **A note on the staining of tubercle bacilli in sections.** *J. Lab. & Clin. Med.*, **23**, 416–8. 1938.

The method gives satisfactory results after formalin and Zenker-formol fixation. With formalin, the green counterstain fades in a few months but bacteria and nuclei remain well stained indefinitely. With Zenker-formol all structures retain the dyes for prolonged periods. Sections of all viscera, brain, and meninges stain well and it is particularly suitable for bone marrow studies and for microphotography.

Procedure: Rinse slides in dist. water; mordant in 5% Fe alum for 5 min. at 45°–50° C.; rinse, and stain 5 min. at 45°–50° in following hematoxylin: dissolve 1 g. Grübler's hematoxylin in 80 cc. of hot dist. water, cool, add 10 cc. glycerin and 10 cc. 95% alcohol; differentiate in picric acid (sat. alc. sol. picric acid and 95% alcohol, 2:1) for 5 min. or until stain remains only in nuclei; wash thoroly in running water; flood with fuchsin (16 cc. sat. alc. sol. basic fuchsin in 84 cc. anilin water), and heat until steaming. Cool for 3 min., heat again, cool for 3 min.; destain shortly in acid alcohol (3% nitric acid in 95% alcohol); rinse in tap water and continue destaining until sections are barely pink; rinse, and dip into alkaline water (NH_3 water) for a few seconds; wash in running water for 10 min. or more; stain 5 min. in 1% light green (Grübler). Rinse in water and run up quickly thru the alcohols and xylene. Mount as usual.

The nuclear membrane is dark blue green; the nucleoli, blue green; cytoplasm paler light green; bacilli bright red.—*A. V. Tolstoouhov.*

HOBBY, G. L. and DAWSON, M. H. **The encapsulation of haemolytic streptococci.** *Brit. J. Exp. Path.*, **18**, 212–4. 1937.

Preparation of culture: Neopeptone beef infusion agar containing 0.2% dextrose and 2.5% rabbit's blood is used. Freshly poured plates are essential in order to insure an adequate amount of moisture. The dextrose is added to the agar at the same time as the blood. The organisms are grown on this medium either for 3–5 hr. at 37° C., or for 10–12 hr. at room temp.

Preparation of reagents: A 4.8% solution of alcoholic (95% C_2H_5OH) gentian violet is kept as the stock solution. For use, 5 cc. of this solution is added to 95 cc. of dist. water, and the final solution then passed thru filter paper. *Method of staining:* Make a thin smear by emulsifying the organism in a loopful of serum; allow to stand for 10–15 sec. and then spread over the slide. Dry in air and fix gently with heat. The slide should never be heated enough to warm it appreciably. Flood with gentian violet and heat until the dye just begins to steam, but no longer. Wash quickly with a small amount of 20% copper sulfate and dry by blotting. Care should be taken that the slide is completely dry within 5 sec. after the copper sulfate is first applied. The foregoing procedure is essentially the same as the well-known capsule stain described by Hiss in 1905. In employing this technic, however, it is important to use as little heat and as little copper sulfate as possible. The capsules on haemolytic streptococci are more readily destroyed by both these factors than are those on pneumococci. By this procedure distinct and well-defined capsules have been demonstrated on the mucoid phase of haemolytic streptococci belonging to Groups A to F inclusive.—*J. A. Kennedy.*

HISTOCHEMISTRY

GENDRE, H. **A propos des precédés de fixation et de détection histologique du glycogène.** *Bull. d'Histol. Appl.*, **14**, 262–4. 1937.

The author believes much of the difficulty in glycogen staining is due to insufficient fixation. He recommends fixing ¼ hr. in: 8 vol. of 90% alc. saturated with picric acid; 1.5 vol. of formol; 0.5 vol. of glacial acetic acid. This fixation may be followed by either alcohol-xylene-paraffin, or by dioxane-paraffin embedding. Plunge the deparaffinized sections into a Barrel tube filled with dist. water to which has been added 25 drops of an acidified sat. aq. solution of anilin blue. Counterstain with Lugol's. Differentiate in iodized alcohol. Mount in mineral oil. The glycogen stains a mahogany brown. Results were checked by using Best's carmine stain, Mayer's mucicarmine, the Schiff reaction and salivary digestion.—*M. Lois Calhoun.*

KREMER, J. **Zum mikrochemischen Eisennachweis im Leber- und Milzpigment der Kaltblüter.** *Zts. Wiss. Mikr.*, **54**, 429–32. 1938.

Amphibia and reptiles that spend the winter months hibernating offer excellent material in their liver and spleen for the study of aggregation of pigments, and the deposition of Fe. The two principal pigments found, one rather coarse and yellowish and the other finely granular and brown-black, seem to be related to Fe, in the detection of which the following technic is employed: Fix preferably in abs. alcohol. Cut 10 μ paraffin sections. Bleach in 3–5% H_2O_2 for 3–5 days. Wash thoroly in dist. water and transfer into either K ferrocyanide and HCl (Berlin blue reaction), or K ferricyanide and HCl (Turnbull blue reaction), preceded by a short darkening in $(NH_4)_2S$. The blue coloration demonstrates that both pigments contain Fe.—*J. A. de Tomasi.*

VERNE, J. **Considerations sur les états histochemiques des lipides.** *Bull. d'Histol. Appl.*, **14**, 269–78. 1937.

The work was carried out on dog kidney and myelin sheath to determine whether certain reactions were due to oxidation or to the presence of an aldehyde radical. The problem was not solved.

The technic employed was formalin fixation, washing in water, and treatment with sublimate solution. The Feulgen Verne (F.V.) reaction of Lison was negative or only feebly positive before the sublimate treatment, but decidedly positive after it. Osmic acid and chlorides of metals with atomic weights between 191 and 201 (Ir, Pt and Au) in concentrations of 0.5–1% could be substituted for the sublimate. Salts of heavier or lighter metals were without action. Strong oxidizing agents, such as H_2O_2, $KMnO_4$ and $K_2Cr_2O_7$, gave a positive reaction only after several days. The negative reaction produced by the action of formalin alone may be offset by using 1 part in 3 of 1% solution of the active metals. After treatment by Feulgen reagent the sections were rinsed in 3 or more baths of bisulfite solution (H_2O, 1 liter; commercial liquid sodium bisulfite, 50 cc.; and *N* HCl, 50 cc.). This deepens the violet tint. Staining may be completed with Sudan, osmic acid or Del Rio Hortega's ammoniacal silver technic. The author found that the lipoidal inclusions show the following characteristics: they reduce osmic acid, fix Sudan and the carotinoid pigments; the F.V. reaction is negative; and the nadi indophenol blue reaction is either negative or colored. The F.V. reaction may be positive if the other reactions are all either positive or negative. Myelin shows an intense F.V. reaction, a slight Sudan fixation and osmic acid reduction, is negative to nadi, and the carotinoids are not fixed.—*M. Lois Calhoun.*

WEBER, F. **Silber-Reduktion der Chloroplasten, Sammelreferat.** *Protoplasma*, **29**, 426–33. 1937.

A review article discussing the significance of the reduction which occurs only in living chloroplasts with the indication that the reduction of acetic acid silver nitrate is a cytological demonstration of vitamin C or ascorbic acid.—*R. Chambers*

STAINS RECENTLY CERTIFIED

In the table below is given a list of the batches of stains approved since the last one listed in the April number of this Journal.

STAINS CERTIFIED MARCH 1, 1938 TO MAY 31, 1938*

Name of dye	Certification No. of batch	Dye Content	Objects of tests made by Commission†	Date approved
Sudan IV	CZ–3	67%	As fat stain	Mar. 3, 1938
Hematein	FHi–3	——	As histological and cytological stain	Mar. 10, 1938
Wright's stain	CWr–11	——	As blood stain	Mar. 14, 1938
Crystal violet	LC–13	96%	As histological, cytological and bacteriological stain	Mar. 25, 1938
Malachite green	NMg–6	98%	As histological and bacteriological stain	Apr. 4, 1938
Carmine	NCa–6	——	As histological stain	May 6, 1938
Congo red	NQ–7	91%	As histological counterstain	May 9, 1938
Wright's stain	LWr–8	——	As blood stain	May 16, 1938
Sudan III	CY–4	82%	As fat stain	May 31, 1938
Basic fuchsin	LF–4	93%	For general staining, the Feulgen reaction, and in bacteriological media	May 31, 1938

*The name of the company submitting any one of these dyes will be furnished on request.

†It is not to be inferred that these are the only uses for which each of these samples may be employed. The Commission ordinarily tests each dye for such of its common uses as seem to give the most severe check as to its staining value. Certification does not in any instance, however, imply approval for medicinal use